3 1761 06705042 7

A MANUAL

of

HISTOLOGICAL PHARMACOGNOSY

AND

BACTERIOLOGY

DARBAKER

With best wishes to

W. S. Wallace

Lyt Dorland.

Jan 15-26.

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ERATA

Index Add Aloe 122.
Diphtheria 339 to 344.
Hamamelidis Cortex 20.
Haematoxylon 155.
Jenner 244, 345.
Plencisz.
Strophanthus 83, 87.
Quercus 22.

Pg. Ln.
8, 18—Mucilagenous taste, foenugreek odor, Ulmus (N. F.)
9, 13—Shaken with sodium hydroxide solutions give a red color.
10, 20—give a blue-black color (similar to Rheum), while Viburnum gives a green color.
95, 7—minutes, transfer to 8% ammonia water for three minutes.
Page 115—MILK CONTAINS IN 100 PARTS.
Line 18 to 35.
Milk serum 91.7
Sodium 0.07
Fat, soluble 1.6
Lime 0.14
Potash 0.175
Chlorine 0.1
Phosphorous 0.17
Butter fat 3.6
Magnesia 0.017
Milk protein 4.0
Milk protein 4.0
Sulphur 0.27
Iron 0.001
Citrates 0.1

Pg. Ln.
124, 19—Delete—Pale yellow, oily, peculiar odor, Oleum Adeps. N. O.
125, 5—manganate solutions, an odor of benzaldehyde develops.
125, 7—no odor, as no cinnamic acid is present, on sublima.
134, 30—diagnostic end cell collapsed trichome,
138, 44—but cells of the phloem and xylem.
141, 14—2. In monoclinic prisms.
161, 35—In 400 B. C. Hippocrates, the father of medicine, believ-
162, 24—perfected by the "father of bacteriology" Leeuwenhoeck, in 1667.
34—Kircher in 1659 suggested that diseases might be due to
39—In 1667 Spallanzani boiled fermentable liquids in a tube
44—Plencisz found certain bodies in diseases and claimed
163, 14—Schulze in 1836 prevented fermentation and decay in
32—Pasteur, a French chemist, was born in 1882, educat-
46—1872. Cohn, classified micro-organisms as to their shape.
180, —Transfer last two lines.
181, 33—requires 1,200 grams to every 1,000 cubic feet.
186, —Delete lines 32 to 37 and insert to book, also delete the words—the co-efficient—on line 38.
200, —Transfer the headings ADAPTABILITY and DANGEROUSNESS.
223, 20—pig against the L plus dose (The L plus dose
243, 2—days, is an antitoxic unit.
288, 29—Stains for the Bacillus Diphtheria. (Caps)
291, 19—grams, water 500 mils, heat and cool; then carefully add com-
20—mercial sulphuric acid 80 mils; dissolve in 500 mils water; place slides and covers in
300, 37—sorbent base containing the moist living yeast cells. Yeast
303, 4—cidious or milk teeth, numbering 20. The second set, or the
315, 5—resembles leukocytes but coarser and larger-pavement
365, 20—ple tint. Then add a 10% sodium sulphite solution un-
22—25 mils to the L.)
366, —Replace table by, insert page.
425, —Insert page.
484, 3—water or saline solution so that it contains not over 20%
488, 8—Pharmacist.

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M Pha.
D

A MANUAL

of

HISTOLOGICAL PHARMACOGNOSY

AND

BACTERIOLOGY

By

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Head of the Department of Pharmacognosy and Bacteriology of the
Pittsburgh College of Pharmacy, University of Pittsburgh.

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To

FATHER *and* MOTHER

This Book is

Affectionately Dedicated

PREFACE

This work owes its existence to the desire of the author to place a concise manual in the hands of those taking the courses in Histological Pharmacognosy and Bacteriology, in the Pittsburgh College of Pharmacy. It is in nowise intended to displace the larger and more elaborate text-books, but aims to present in concise form the essential features of the subjects treated.

The books are the outgrowth of lectures given to students during the past 15 years and also the result of much original work and information gathered from many sources.

The fundamental principles and the laboratory work are outlined as fully as necessary; an expert worker will vary these details as his experience and the conditions may indicate.

The student who wishes to acquire greater knowledge upon any one of the subjects herein treated, will find appended a bibliography on all special subjects included in these manuals.

Volume One forms an introduction to Histological Pharmacognosy and deals with light, lenses and mirrors in their relation to the microscope, the handling and use of the microscope, the various plant cells, tissues, tissue systems and series, cell contents and the structure of the various plant organs.

Volume Two is divided into two parts: The first treats of Histological Pharmacognosy and includes the structure and identification of the various drugs; the second deals with Bacteriology, and includes the examination of blood, urine, feces and related substances, as well as the isolation and cultivation of micro-organisms, and gives information necessary for general laboratory work.

I wish to thank my colleagues, especially Dr. J. H. Wurdack, for help and cooperation in compiling these manuals. Every effort has been made to keep these books within the limits of a manual necessary for students in Colleges of Pharmacy, and to serve as a guide for routine work in laboratories and pharmacies.

PITTSBURGH COLLEGE OF PHARMACY
LABORATORY OF HISTOLOGICAL PHARMACOGNOSY

A text book is not required, although useful. The following books are recommended as text and reference books:

"UNITED STATES PHARMACOPOEIA, (IX)."
"SQUIBB'S ATLAS OF OFFICIAL DRUGS," by Mansfield.
"MEDICAL WAR MANUAL NO. 5." (Laboratory Methods of the U. S. Army).
"GENERAL BACTERIOLOGY," by.....Jordan.
"BACTERIOLOGICAL METHODS OF FOODS AND DRUGS," by .....Schneider.
"Elements of Vegetable Histology," by.....Base.
"Microscopy," by .....Cross and Cole.
"Pharmacognosy," by .....Joliffe.
"Kraemer's Pharmacognosy."
"Leitfaden Zu Mikroskopisch-Pharmacognostischen Ubungen," by .....Miller.
"Materia Medica and Pharmacognosy," by.....Culbreth.
"Organic Materia Medica and Pharmacognosy," by Sayre.
"Microscopy of Vegetable Foods," by.....Winton.
"Fungus Diseases of Plants," by.....Dugar.
"Microscopical Examination of Foods and Drugs," by ..... Greenish.
"The Microscope in Medicine," by.....Beal.
"Plant Anatomy," by.....Steavens.
"Pharmaceutical Botany," by.....Younkin
"Experimental Pharmacology," by.....Green.
"Clinical Chemistry, Microscopy and Bacteriology," by.....Klopstock and Kawarsky.
"Manual of Clinical Diagnosis," by.....Simon.
"Bio-Chemical Drug Analysis," by.....Pittinger.
"Infection, Immunity and Serum Therapy," by.....Rickets.
"Principles of Bacteriology," by.....Abbot.
"Pathological Bacteriology," by.....McFarland.
"Laboratory Bacteriology," by.....Frost.
"Clinical Haematology," by.....DeCosta.
"Practical Urinary Analysis and Diagnosis," by.....Purdy.
"Urine and Faeces in Diagnosis," by Hancil, Weil and Joliffe.
"Milk Bacteriology, Practical Dairy Bacteriology," by Cohn.
"Micro-Organisms of the Human Mouth," by.....Miller.
"Micology of the Mouth," by.....Gadby.
"Practical Bacteriology, Blood Work and Parasitology," by ..... Stitt.

"Elementary Chemical Microscopy," by.....Chamot.
"Microscopical Analysis of Metals," by.....Osmond and Stead.
"Standard Methods of Water Analysis," by.....A. P. H. A.
"Clinical Diagnosis," by .....Todd.

EQUIPMENT TO BE FURNISHED BY EACH STUDENT

The student must keep the following equipment in good condition and must replace the materials used or destroyed: Section Cutter—to be always kept sharp and in good cutting condition; Slide Forceps; Pair Small Scissors; Pincettes; Box of 100 Slides; Slides and Cover Glasses; Two Teasing Needles; One Small Beaker; One Small Evaporating Dish; Lens Paper; Blotters; Labels; Glycerin Jelly; One Small Iodine Brush; Piece Old Linen for cleaning your microscope; One Dish Slide; Platinum or Ni-chrome Needle three inches long, mounted in a glass handle; small Razor Hone.

OUTLINE FOR MATERIA MEDICA LABORATORY

Name..... Seat.....

Date.....

Official name of drug.
English name of drug.
Source and family.
Condition.
Habitat.
Synonym.
Part of plant used.

INSERT DESCRIPTION NO. 1, 2 OR 3

No. 1. FOR ROOTS, RHIZOMES AND BARKS.
- Color—Internal and external.
- Fractures.
- Periderm.
- Peculiarities.

No. 2. FOR FRUITS AND SEEDS.
- Texture—Outer and inner layers.

Nature of contents.
Color—Outer and inner.
Surface.
Peculiarities.

No. 3. FOR LEAVES, FLOWERS AND HERBS.
Petiolate.
Shape—Outline.
Base.
Apex.
Margin—Entire, serrated, et al.
Surface—Hairy, smooth, et al.
Texture.
Venation.
Odor.
Taste.
Constituents.
Medical properties.
Average dose.
Toxicology—Symptoms, antidotes, etc.
Commercial.
Collecting.
Preserving.
Adulterants and detection.
Remarks.

BARKS

The drug names in CAPITALS indicate U. S. P. drugs; those in small letters the drugs of the N. F., and those marked N. O. are not official.

The term Bark or Phloem indicates all that portion outside the cambium zone.

With Periderm.
1. Yellow to red brown in color.
A. Short fracture.
a. In quills.
1. Characteristic odor and taste.
Dark brown, CINNAMONUM SAIGONICUM.
2. No characteristic odor or taste.
Red brown, few lenticels, bitter,
CASCARA SAGRADA.
Red brown, fissured, fibrous, resinous, Paracoto.

Gray brown, granular, inner surface striated,
Condurango.

b. Transverse curved or flattened pieces.

Purple gray, with black dots, lenticels, brittle,
FRANGULA.

Gray black, valerian like odor,
VIBURNUM PRUNIFOLIUM.

Red brown, gray cork, astringent,
Hamamelidis Contex N. O.

Yellow brown, odor, Chionanthus.

Dark brown, inner surface purple, yellow stone,
Cornus.

B. Fibrous fracture.

Red brown, coarse and fibrous, Cossypii Cortex.

Gray brown, odor, Cocillana.

Gray brown, fissured, grouped white stone,
ASPIDOSPERMA.

Gray brown, black headed white lichens, musk odor on warming, Cascarilla.

Pale brown, warty ridges, Fraxinus.

Brown black, astringent, coarsely fibrous, Rubus.

2. Gray white to gray black.

A. Flattened or transversely curved.

a. Short fracture.

1. Crystalline inner surface.

Black headed white patches, tinging taste,
XANTHOXLYUM, Northern.

2. Inner surface not crystalline.

Conical cork rings, XANTHOXYLUM, Southern.

Conical depressions, thick, fissured, GRANATUM.

b. Fibrous fracture.

Inner surface fawn color, silky fracture, Euonymus.

Inner surface whitish, uneven fracture,
Viburnum Opulus.

Red broken cork, root bark, Myrcia.

3. Greenish in color.

Flattened or transversely curved.

a. Short fracture.

Stem scars, fissures, on chewing bitter almond taste,
PRUNUS VIRGINIANA.

Fibrous fracture.

Greenish, silky white bast, MEZEREUM.

No Periderm.

1. Yellow red or brownish.

A. Quills.

a. Short fracture.
Granular fracture, odor and taste, CINNAMOMUM ZEYLANICUM.
Uneven fracture, warty, inner surface smooth, striated, odor and taste aromatic, no cork, Juglans.
Brown orange, on burning cinnamon odor, Canella.

B. Flattened or transversely curved.
a. Short fracture.
Mottled red, no cork, odor and taste, SASSAFRAS.
b. Fibrous fracture.
Pitted surface, turpentine odor, astringent, Pinus Alba.
No cork, coarsely fibrous, astringent, Quercus.

2. Yellow white.
A. Flattened pieces.
a. With crystals.
Sternutatory, both surfaces crystalline, QUILLAJA.
b. No crystals.
Mucilaginous taste, fenugreek odor, ULMUS.

BARKS OR PHLOEMS

The tissues beneath the, or with the epidermis and outside the cambium zone constitute the bark.

Barks are richer in stored contents than woods; the root bark being the richest, but a separation of bark is not practical in many cases; hence roots and rhizomes are used in medicine.

The typical bark is composed of three parts:

The "endophloem"—inner bark is the most active in growth, being next to the cambium, usually with sieve cells, which may be obliterated, and sometimes containing bast fibers—Parenchyma predominates.

The "mesophloem"—middle bark—is richest in stored products, consisting of collenchyma, parenchyma, bast and stone cells, sometimes laticiferous.

The "epiphloem"—outer bark, or "bork"—consists of the outer protecting cells, epidermis, and cork. Some barks may have a "Hypoderm". When the epidermis is replaced by cork, the epiphloem is known as the "periderm".

The contents of the barks are starch in varying amounts, according to the season of collecting, alkaloids, oil, resins, mineral compounds, secretions, et al.

The cells found in a typical bark are, epidermis, cork, phellogen, collenchyma and parenchyma—scattered through

the collenchyma and parenchyma are bast, stone, laticiferous and sieve, usually obliterated—sometimes crystal bast and reservoirs.

Barks are best studied in the longitudinal and transverse sections. Remember that typical bast cells do not give the ligno-cellulose reaction, but in most of the official drugs they are lignified. Bast and stone usually are found in the mesophloem, as are also the soft bast and vessels.

Diagnostic points of some of the barks:

Sections of Cascara Sagrada turn orange with alkalies, no cork proper, stone, crystal bast, rosette crystals of calcium oxalate, medullary rays two cells in width. The powder shaken with sodium hydroxide solutions give a brown color. Frangula gives a red foam. Many medullary rays, not grouped but single and straight; the cambium is even.

Sections of Rhamnus Californica turn red with alkalies, cube and rosette shaped crystals, typical irregular crystal bast. The medullary rays are few. They are curved and grouped. The cambium is wavy.

Frangula contains rows of thick lignified bast, calcium oxalate crystals. No stone. The powder shaken with soddium hydroxide solutions gives a red foam.

Rhamnus cathartica, Rhamnus carniolica, Frangula, and Cascara Sagrada contain no anthraquinone derivatives in the cork, having instead, a red brown content. Inner cells of Rhamnus cathartica, and Rhamnus carniolica contain this substance. In fresh Frangula little or none of this substance is found, only in the older. The fractures of Frangula and Cascara are smooth, that of Rhamnus carniolica, short and fibrous—while that of Rhamnus cathartica is long and fibrous.

Cinnamomum has characteristic lop-side stone cells, short bast, collapsed sieve cells with acicular crystals, oil cells.

Quercus appears with the rings of sclerosed bast and stone, no cork and much tannin.

Granatum appears as tangential rows of cells with rosette crystals of calcium oxalate, starch, single large stone cells, crystal bast, sphenoidal micro-crystalline cells and differing from other barks by when dipped in water and rubbed on white paper, and to the stain is added ferric sulphate solution a yellow to blue color is produced; if nitric acid is now added the stain becomes rose red.

Cinchona has spindle shaped bast tannin, oil, few or no stone, little starch. Cinchona Calisaya contains few or no stone—the bast is single, or in twos. Cinchona succirubra

contains no stone, the bast is grouped from two to five, seldom over eight.

Maracaibo bark contains many stone cells.

Powdered Cinchona heated in a test tube will give a granular purple distillate. Cinchona Rubra gives a bright red—The more alkaloids contained in the bark, the brighter red the distillate.

Quillaja contains calcium oxalate crystals in lumens of the very thick walled lignified bast, clumps of saponin, which with sulphuric acid gives a yellow solution, turning to red, finally to violet.

Viburnum Prunifolium has few or no bast—bast replaced by stone, calcium oxalate crystals.

Viburnum Opulus has few or no stone—bast replaces stone, rosette crystals.

Sections of Acer Spicatum (Mountain maple—an adulterant for the Viburnums) with ammonia gives a crimson color. This reaction is similar to the emodin reaction of the cathartic drugs. Sections with a solution of ferrous sulphate give a blue color (Similar to Rheum).

The tannin of the Viburnums, with a fresh solution of ferric chloride, gives a green color; while the tannin of Acer Spicatum will give a blue color. Sections of the Viburnums, with solutions of phloroglucin and hydrochloric acid, give a little red color, while those of Acer Spicatum give an intense red color. The tannin in these drugs is found in the parenchyma and medullary cells. The Viburnums have rosette crystals; Acer Spicatum has prismatic crystals and crystal bast.

Chionanthus has thin walled cork, parenchyma with two to four compound starch, small stone single or grouped, with branched pores.

Rubus cuneifolius has bast containing starch grains.

Hamamelidis Cortex irregular outlined stone, narrow crystal bast, tannin and oil.

Sassafras Cortex has little or no cork, many mucilage and oil cells, characteristic thick walled, lignified bast, tannin.

Ulmus has no cork, long slightly lignified bast, a few crystal fibers, a little starch and much mucilage.

Condurango has phellogen with starch, thick walled non-lignified bast, many stone, rosette crystals.

Euonymus shows thin walled non-lignified bast, secretion cells, rosette crystals, bast; medullary rays and parenchyma contain a yellow brown content.

Xanthoxylum shows many oil receptacles, few starch or crystals, non-lignified bast, stone cells with reddish brown content; some parenchyma cells are lignified. The Southern has rings of lignified cork, large stone, scattered bast, many starch and some monoclinic crystals, in the parenchyma and a few crystal bast.

Aspidosperma has characteristic clusters of stone.

Gossypii Cortex contains bast, tannin, cells with reddish contents.

Mezereum contains long silky non-lignified bast, square in transverse view.

EXERCISE

Make two longitudinal sections and two transverse sections; stain one set with the ligno-cellulose stain; and stain the other set with the cellulose stain. Look for the characteristic points; make drawings before removing cell contents. REPORT.—1. Draw under the low power, then with the high power fill in the cells. Name ALL cells. Make drawings of both sections. 2. Is bast present, or replaced by stone? 3. Draw the bast and stone. 4. Are they lignified or not? Is much, little or no cork present? 6. Draw and name the characteristic cells. 7. Are crystals present? If so, name the kinds, shapes, and give tests. 8. Is starch present or not? Note markings and shape; draw. 9. Are crystal fibers present or not? If present, draw. 10. Are oil, mucilage or other cell contents present? If so, give tests and reactions. Answer the above for each bark studied. After finishing each bark, ask for the powder of that bark; use your U. S. P. for the description. Bring your U. S. P. for the next Laboratory period. You cannot get credit if you use another student's books or material. Hand in complete reports.

Several layers of elongated parenchyma, in Prunus Virginiana, Frangula and Cascara Sagrada.

Beaded parenchyma, in Virburnum Opulus.

Bast and Stone in some of the Barks, as follows:

Grouped stone, in Quercus, Cascara Sagrada, Xanthoxylum and Viburnum Prunifolium.

Bands of stone, in Cinnamomum and Condurango.

Isolated stone, in Cinchona, Granatum and Cinnamomum.

Few or no stone, in Mezereum, Ulmus and Viburnum Opulus.

Isolated bast, in Cinchona and Sassafras Cortex.

Grouped Bast, in Frangula, Cascara Sagrada, Cinna-

momum, Sassafras, Cortex, Gossypii Cortex, Prunus Virginiana, Condurango, Euonymus, Xanthoxylum, Quercus, Juglans and Viburnum Opulus.

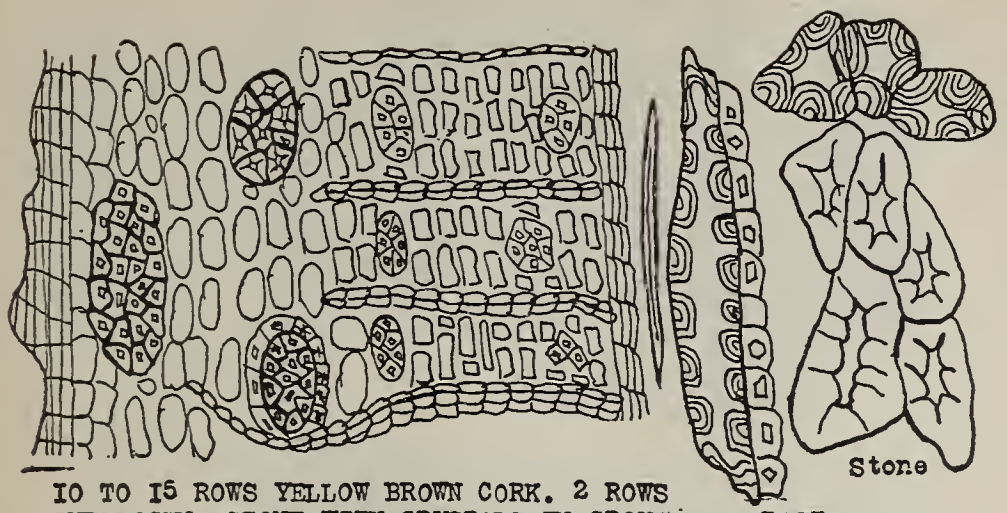
Crystal bast, in Frangula, Cascara Sagrada, Quercus, Granatum, Quillaja, Xanthoxylum and Hamamelidis Cortex.

Longitudinal striated bast, in Cinnamomum, Cascarilla, Cornus, Canella, Sassafras Cortex, Prunus Virginiana, Rubus and Xanthoxylum.

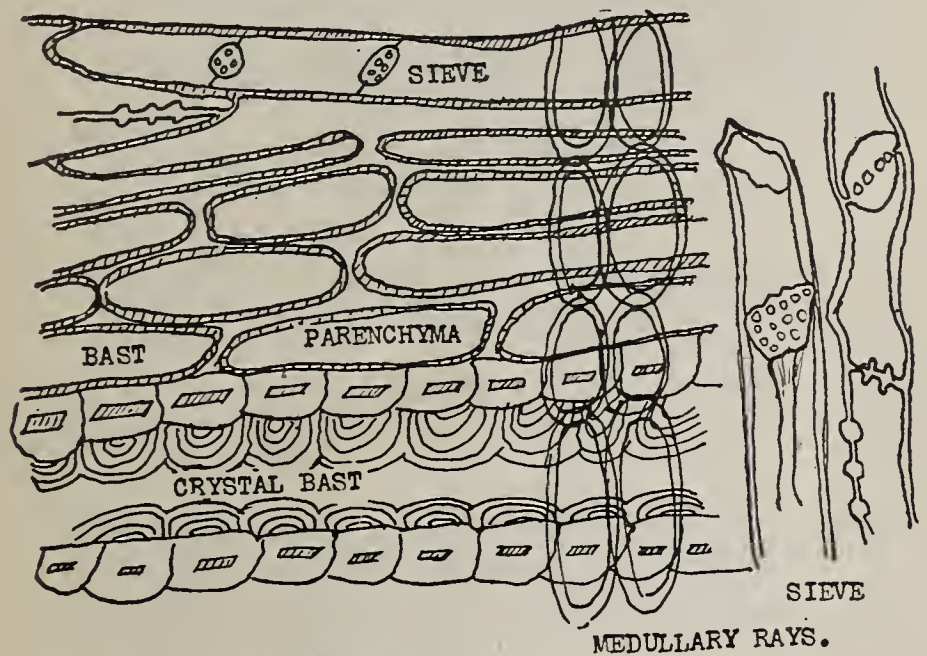
Tangential striated bast, in Aspidosperma, Gossypii Cortex, Mezereum, Euonymus, Viburnum Opulus, Condurango and Juglans.

Quadratically striated bast, in Ulmus, Quillaja, Fraxinus, Juglans and Granatum.

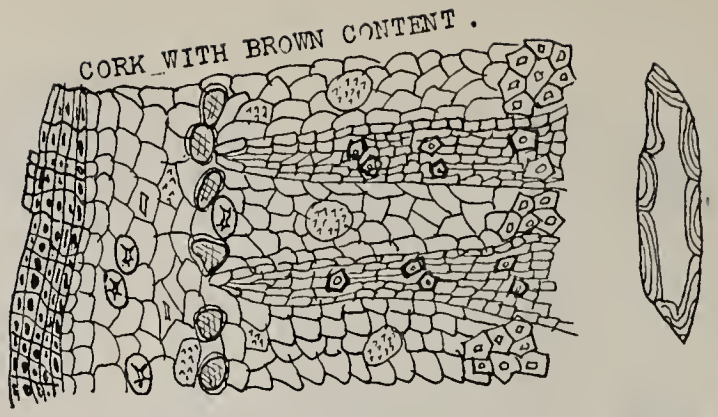
Bast without striations, in Coto, Paracoto, Hamamelidis Cortex, Cascara Sagrada, Frangula, Xanthoxylum, Chionanthus and Granatum.



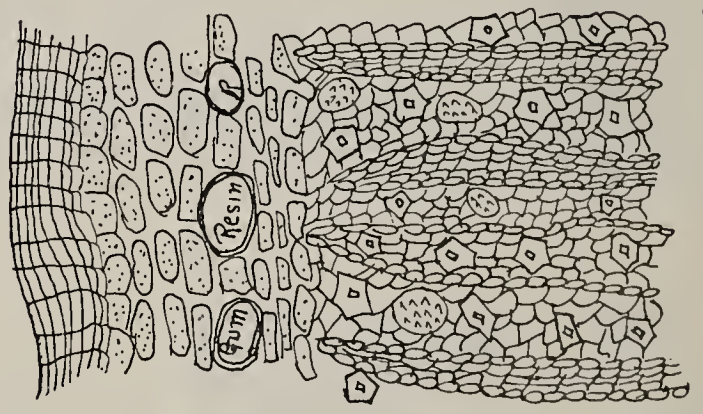
IO TO I5 ROWS YELLOW BROWN CORK. 2 ROWS PHELLOGEN, STONE WITH CRYSTALS IN GROUPS OF I5 TO 20. PARENCHYMA WITH STARCH OR CRYSTALS, ROSETTE OR PRISMS AND WITH A CONTENT WHICH TURNS RED WITH ALKALIES (ALSO THE MEDULLARY RAYS). MEDULLARY RAYS I TO 4 WIDE BY I5 TO 20 IN LENGTH. CRYSTAL BAST AND STONE.



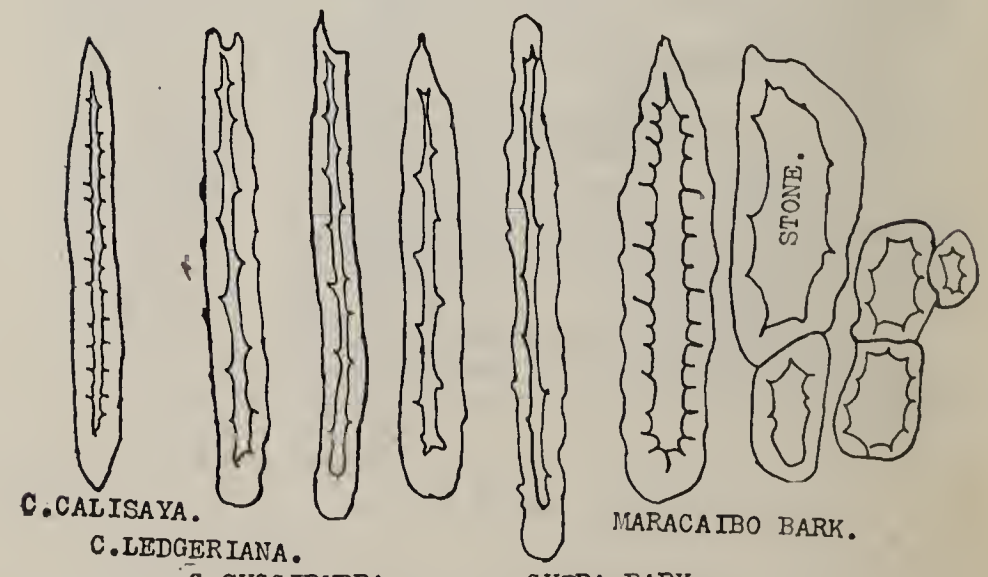
C A S C A R A S A G R A D A .



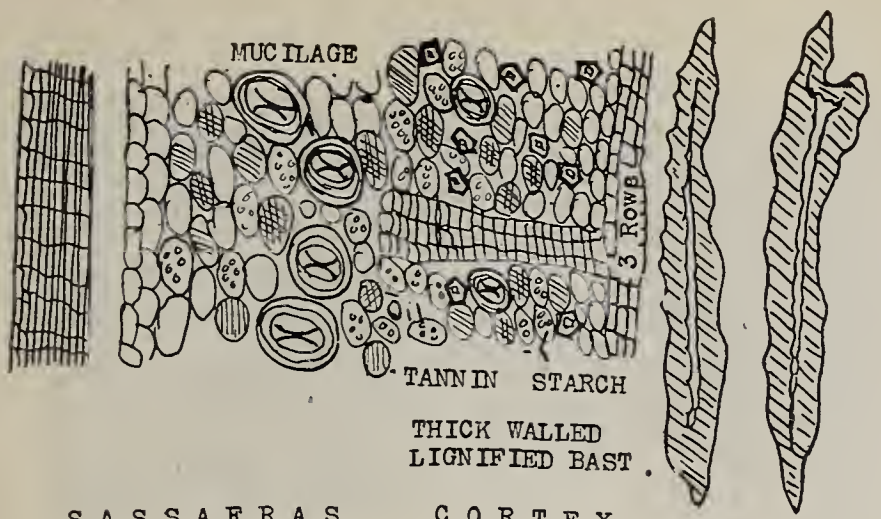
CINCHONA.(CALISAYA)



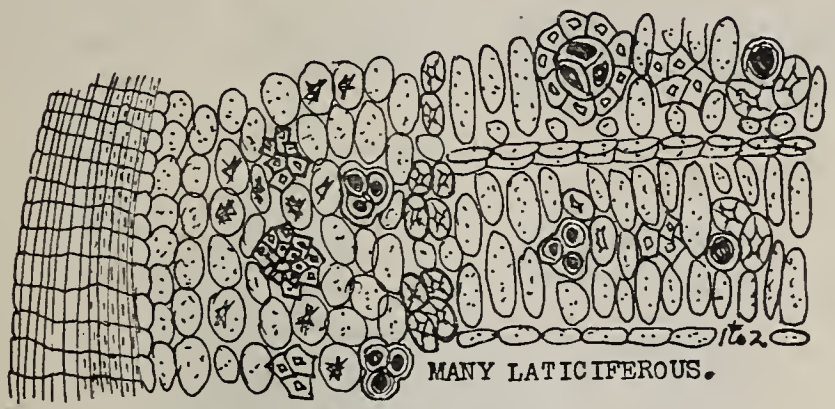
CINCHONA SUCCIRUBRA



C.CALISAYA.
C.LEDGERIANA.
C.SUCCIRUBRA.
C.OFFICINALIS.
CUPRA BARK.
MARACAIBO BARK.



S A S S A F R A S C O R T E X .



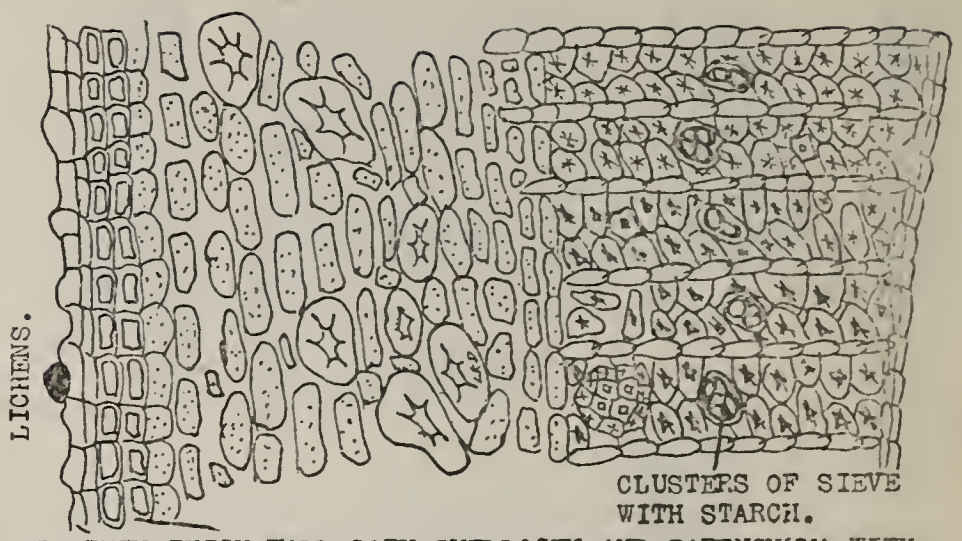
5 TO 10 ROWS THIN GRAY WALLED CORK.
8 TO 10 ROWS PHELLOGEN WITH CRYSTALS AND STARCH.
PARENCHYMA AND COLLENCHYMA WITH CRYSTALS, STARCH
AND CHLOROPLASTS.

THICK WALLED NON LIGNIFIED BAST.

C O N D U R A N G O .

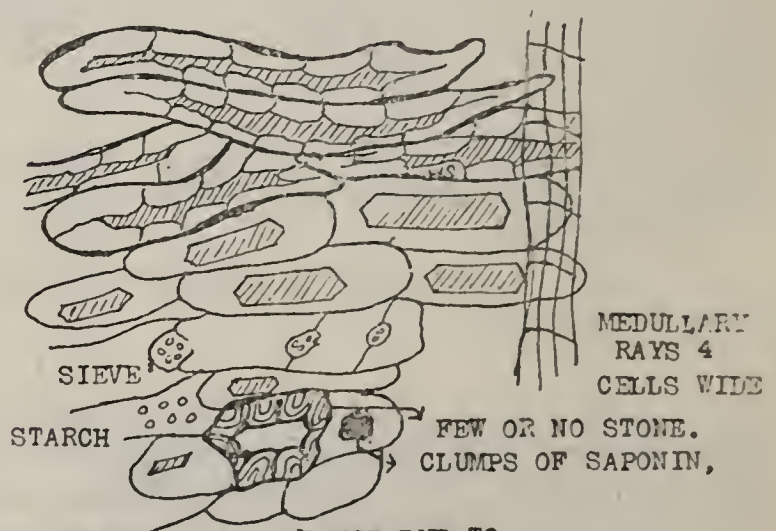
CRYSTALS, STARCH, LATICIFEROUS.
LONG THIN WALLED NON LIGNIFIED BAST.
MANY BAST, PARENCHYMA AND MEDULLARY RAYS CONTAIN
A YELLOW BROWN SUBSTANCE.

E U O N Y M U S .



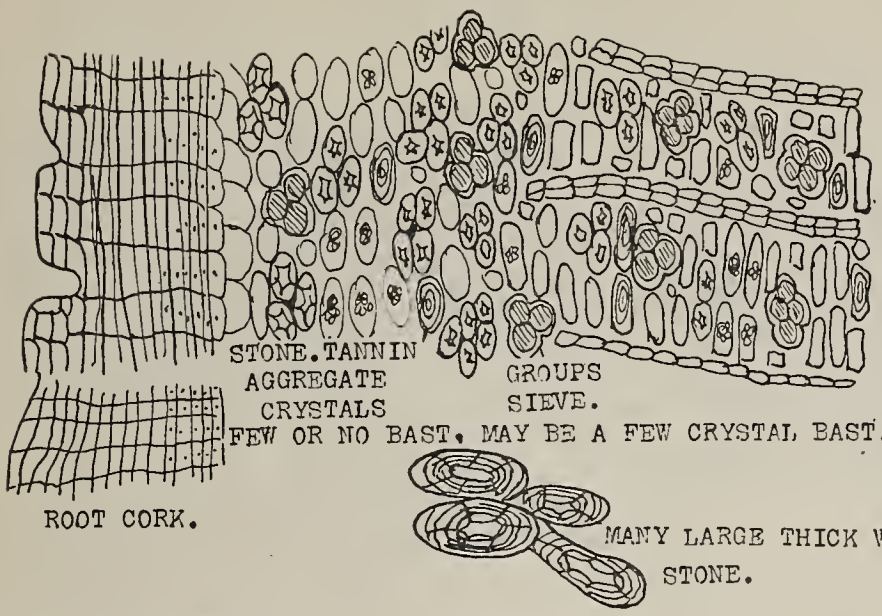
WHITE, THIN, THICK WALL CORK, PHELLOGEN AND PARENCHYMA WITH STARCH AND CHLOROPLASTS, PARENCHYMA ALSO CONTAINS AGGREGATE CRYSTALS AND SPHENOIDAL MICRO-CRYSTALS. A FEW BAST, MAY BE CRYSTAL BAST. LARGE SINGLE STONE.

G R A N A T U M .

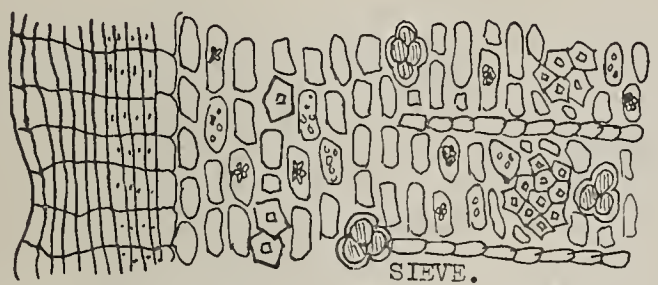


CHECKERED APPEARANCE DUE TO
ALTERNATE ROWS OF SIEVE AND PARENCHYMA.

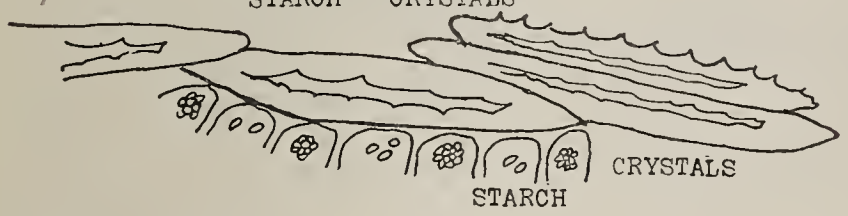
Q U I L L A J A .



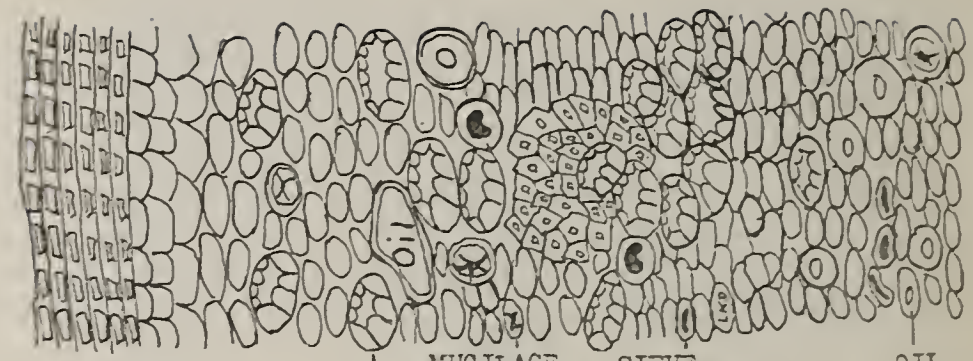
V I B U R N U M P R U N I F O L I U M .



BAST SINGLE OR GROUPED UP TO 10,NON LIGNIFIED.
10 ROWS PARENCHYMA WITH BROWN YELLOW CONTENTS AND
STARCH,CRYSTALS OR CHLOROPLASTS.

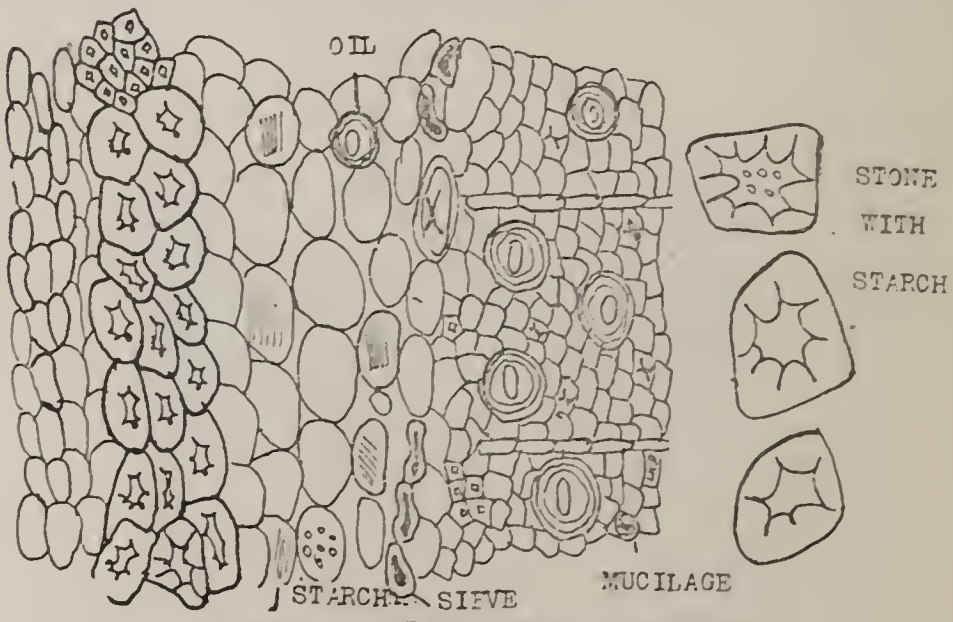


V I B U R N U M O P U L U S .

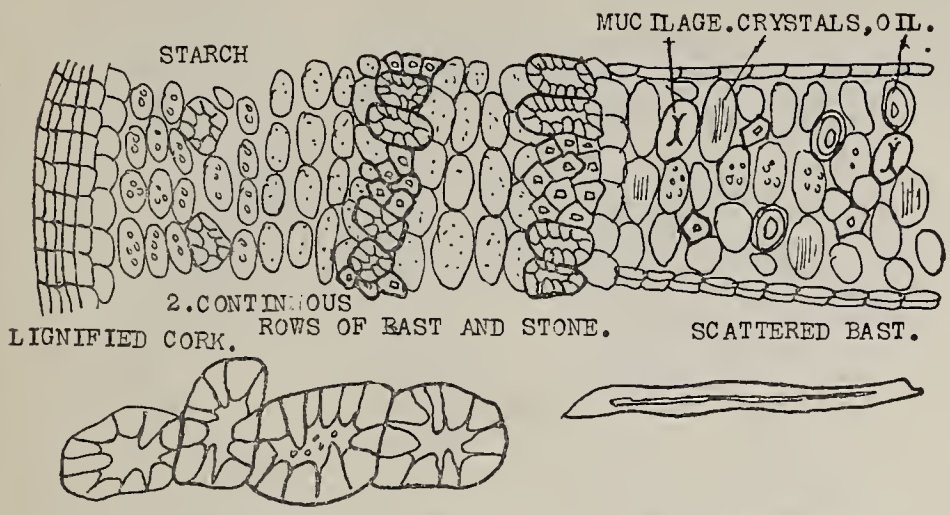


LOP SIDED STONE, IN INTERRUPTED RINGS
PARENCHYMA WITH LARGE PORES.

C A S S I A C I N N A M O N .

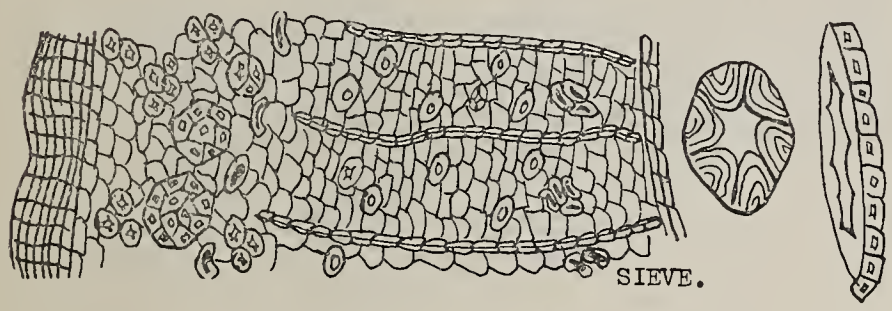


C I N N A M O M U M Z E Y L A N I C U M .



PARENCHYMA,STONE AND BAST CONTAIN A RED-BROWN AMORPHOUS SUBSTANCE.

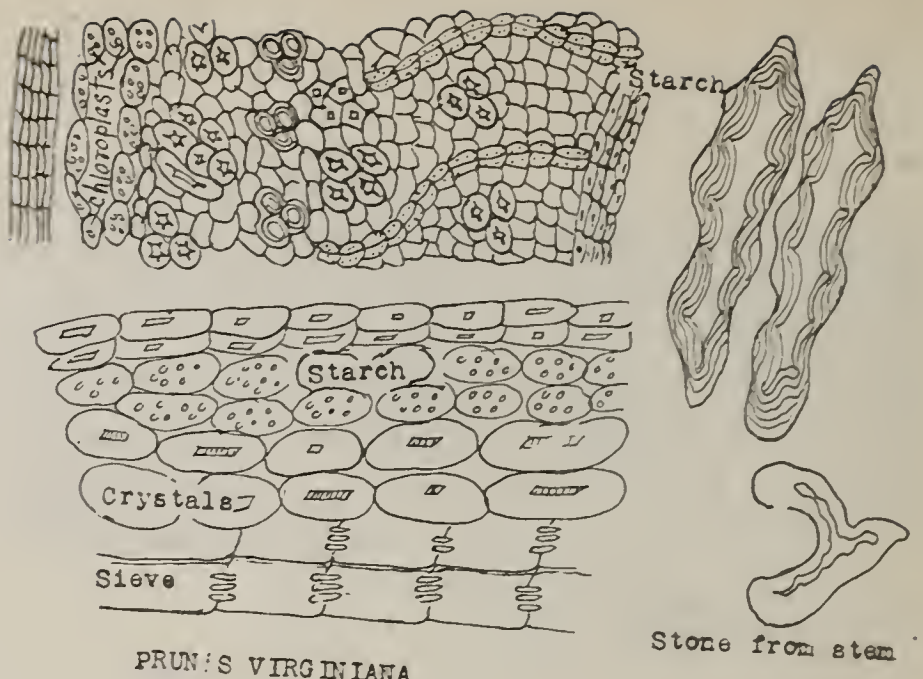
C I N N A M O M U M S A I G O N I C U M .



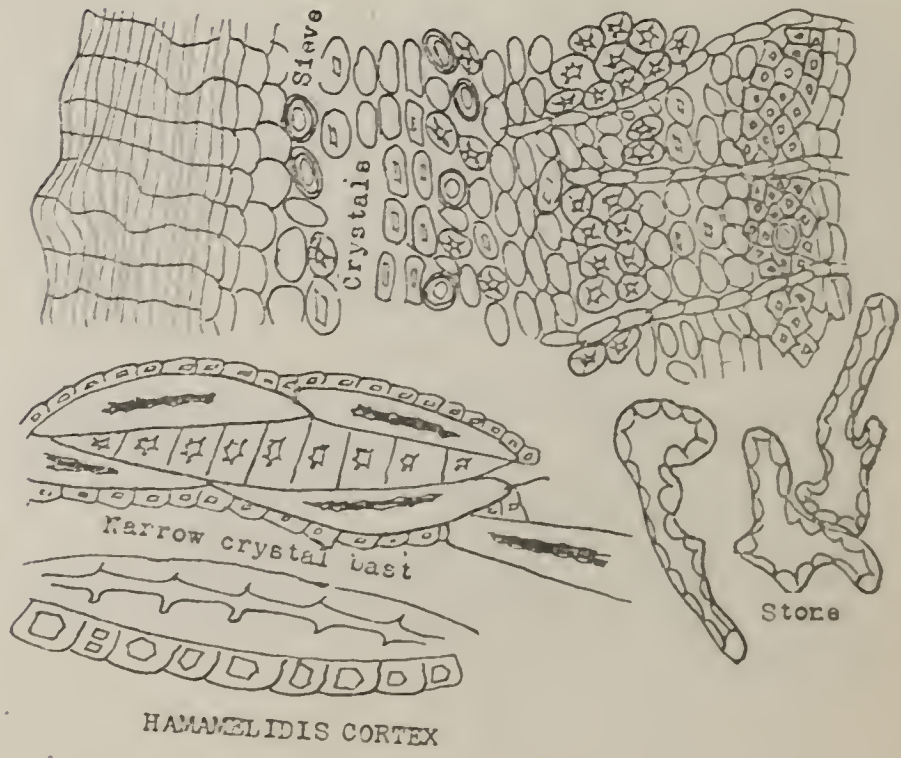
X A N T H O X Y L U M .

XANTHOXYLUM AMERICANUM HAS 8 TO 10 ROWS OF COLLENCHYMA WITH STARCH,CRYSTALS AND CHLOROPLASTS. PARENCHYMA OF THE MESOPHYL ENDO PHLOEM CONTAIN MUCH OIL AND MANY ROD SHAPED OR FLAT CRYSTALS. BAST NON-LIGNIFIED,MEDULLARY RAY CELLS USUALLY ONE CELL WIDE

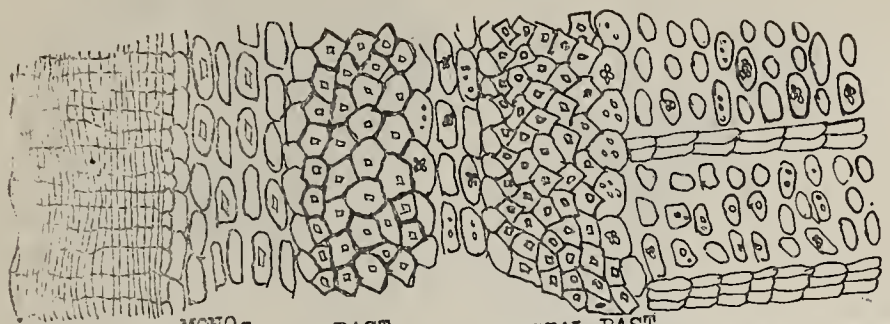
XANTHOXYLUM CLAVA-HERCULIS HAS RINGS OF LIGNIFIED CORK, A FEW ROWS OF COLLENCHYMA,LARGE STONE,SCATTERED BAST,OIL AND SIEVE CELLS,MEDULLARY RAYS ONE TO FOUR CELLS WIDE, MUCH STARCH,MONO-CLINIC CRYSTALS IN THE BAST AND PARENCHYMA.



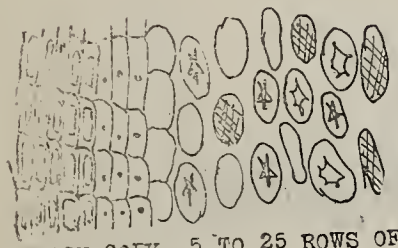
PRUNUS VIRGINIANA

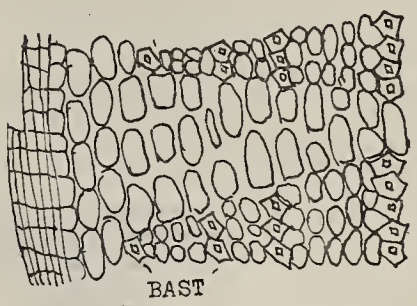


HAMAMELIDIS CORTEX



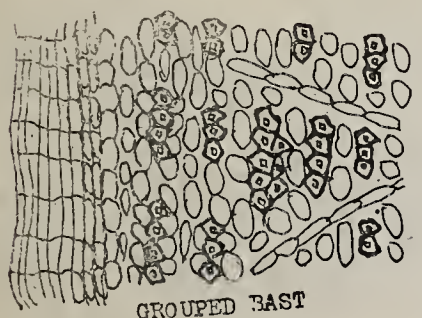
A C E R S P I C A T U M .



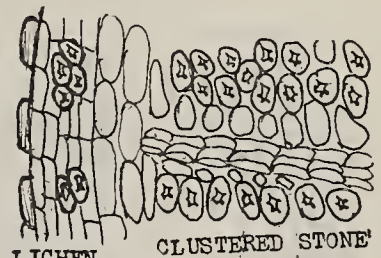


G O S S I P I I C O R T E X .

V I B U R N U M L E N T A G O .



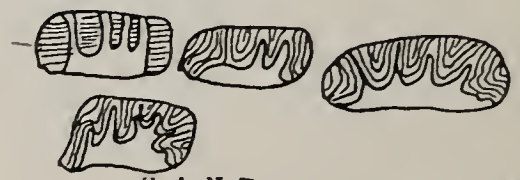
M E Z E R U M .



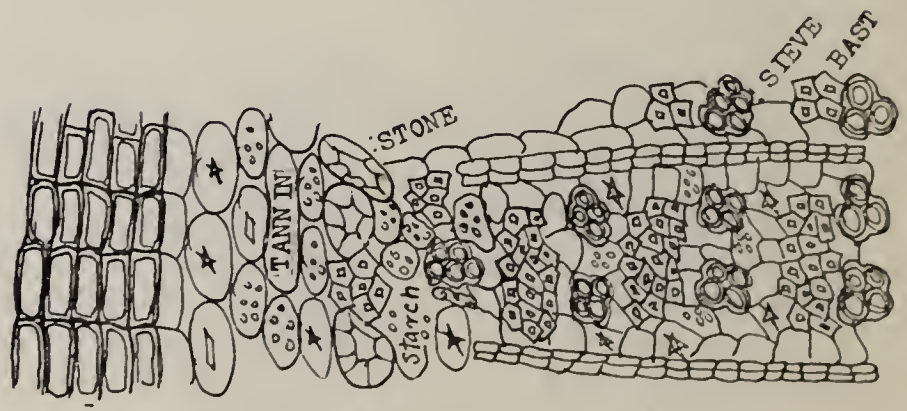
A S P I D O S P E R M A .

QUERCUS.

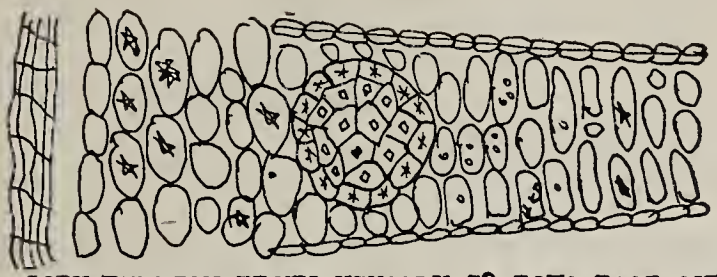
RUBUS CUNEIFOLIUS.



CANELLA ALBA.

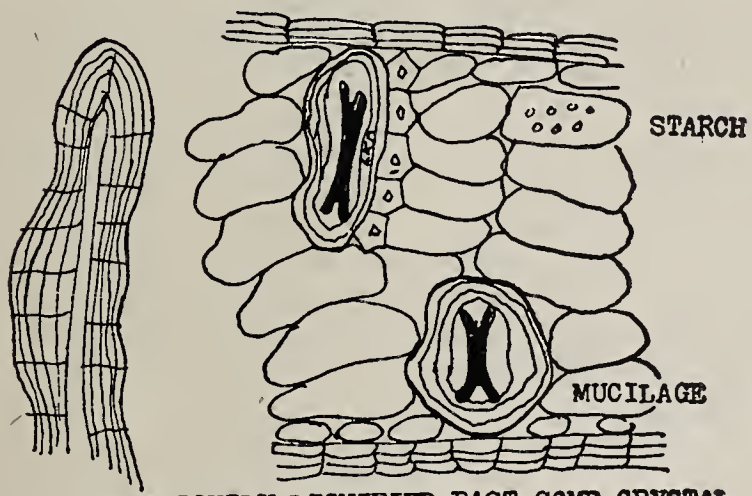


JUGLANS.



CORK REDDISH BROWN, USUALLY I2 ROWS. BAST SURROUNDED BY CRYSTAL FIBERS. PARENCHYMA CONTAINS ROSETTE CRYSTALS AND STARCH.

F R A N G U L A .



LONG SLIGHTLY LIGNIFIED BAST, SOME CRYSTAL BAST WITH MONO-CLINIC CRYSTALS

U L M U S .

WOODS

Wood or Xylem is all that portion enclosed by the cambium zone.

1. Light to bright yellow.
   Odor, Santalum Album
2. Yellow red to yellow brown.
   Chips or small blocks, very bitter, whitish, QUASSIA.
   Violet or wine color with water or saliva, Haematoxylon.
   Purple red, no color to water or saliva, SANTALUM RUBRUM.
3. Green brown to bronze green.
   Heavy wood, sap wood yellow, Guaiaci Lignum.

PITH

Light weight, spongy cylindrical, white yellow, mucilaginous, Sassafras Medulla.

CHARRED WOOD

Dull black fine powder, CARBO LIGNI.

XYLEM, OR WOODS

Such as are enclosed by the cambium zone in the dicotyledons. In the gymnosperms, the wood and tracheae (ducts) are replaced by tracheids with bordered pores (diagnostic of the evergreens). In the monocotyledons the xylem and phloem are together and form the fibro-vascular bundles, and may be pulled out in strings from the fundamental tissue as in corn stalk and celery.

In the dicotyledons the xylem portion forms an interrupted cylinder inside the cambium zone, the medullary rays connecting the pith and cortex. Rings of growth are due to the varying calibers of the cells—largest and lightest in the spring or growing season.

Growth occurring by additions from the meristematic tissue.

The function of the xylem is for support, conduction of air, cell sap and water.

The cells in a typical xylem are the pith cells, medullary rays and cambium zone, all composed of parenchyma cells, wood cells, wood parenchyma, tracheae and tracheids.

Three views are necessary for the full study: Transverse, longitudinal and longitudinal-tangential—so that the ends, sides and lengths of the cells may be seen.

Transverse section shows the tracheae with large calibers, wood cells with small prismatic calibers and very thick

walls—the tracheids resembling wood cells, but with thinner walls and larger lumen, than the wood cells; parenchyma more or less square or oval, thin walled. The other sections reveal to a greater or lesser extent the markings of the cells, their length and their ends (important).

Starches, sugars, calcium oxalate are occasionally found, rarely alkaloids, or nitrogenous bodies. Resins when present as in Guaiacum render sectioning difficult—Tannin and coloring as in Haematoxylon and bitter principles as in Quassia also occur.

Woods are not often adulterated, but are frequently used to adulterate other drugs. Pine sawdust is easily detected by the diagnostic tracheids. Resin and oil secretion reservoirs occur in Pine and Eucalyptus woods.

Guaiacum shows wide vessels, much resin, small heavy wood fibers.

Haematoxylon shows a few crystals, gives a deep red color to water, and is purple with alkalies.

Santalum Rubrum shows medullary rays one cell wide, no color to water or alkalies—wood cells are irregular in outline and porous, with pointed or forked ends and contain a granular content. The vessels are pored and contain a lemon yellow colored resin, a few crystal wood fibers with monoclinic crystals.

Santalum Album shows medullary rays irregular and darker than the wood fibers.

Quassia, the Surinam or amara quassia, contain vessels in groups of three to four, medullary rays one to four cells in width and ten to thirty cells in length, few or no crystals.

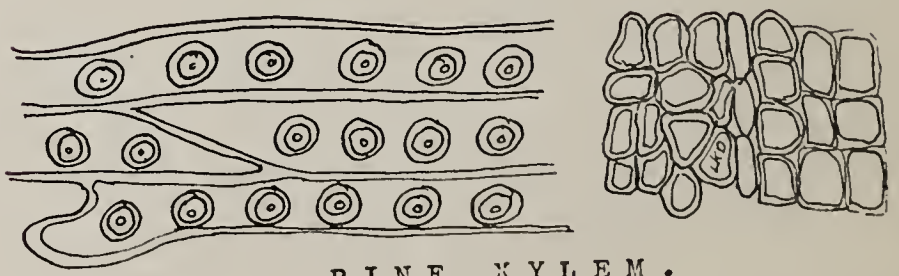
The Jamaica or excelsa quassia contain vessels in groups of two to five, medullary rays one to five cells in width, by ten to twenty in length, many calcium oxalate prisms, four to six sided, few starch, wood cells with thin walls.

Pine xylem may be diagnosed by tracheids with bordered pores.

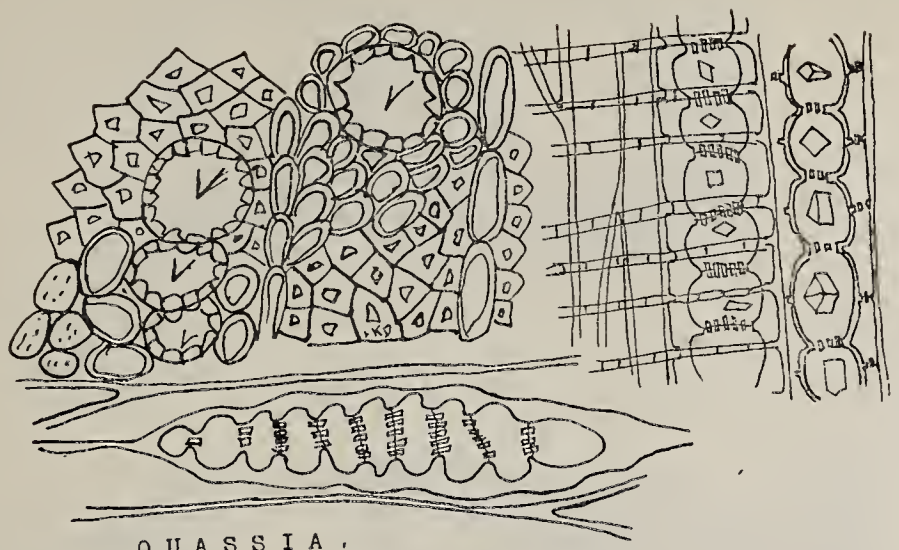
EXERCISE

Make the three sections, transverse, longitudinal and longitudinal-tangential. The wood may have to be softened in Schultze's macerating fluid before sectioning; care must be taken not to soften too much. Note, draw and report for

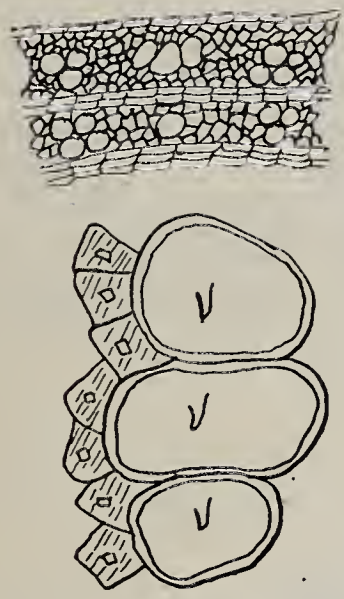
each wood: 1. Draw the section under low power; fill in under high power; note the contents and name all cells. 2. Search for tracheids with bordered pores. 3. Report the size and markings of vessels. 4. Report size, markings and shape of wood fibers. 5. Medullary ray cells and contents. 6. Rings of growth. Use your U. S. P. for the study of the powders. When resin interferes with sectioning or examination, boiling in dilute potassium or sodium hydroxide solutions may remove. KEEP YOUR MICROSCOPE CLEAN. CLEAN IT THOROUGHLY BEFORE RETURNING TO THE CASE. BRING YOUR U. S. P. FOR THE NEXT DAY. READ OVER THE SUBJECT OF ROOTS FOR THE NEXT DAY.



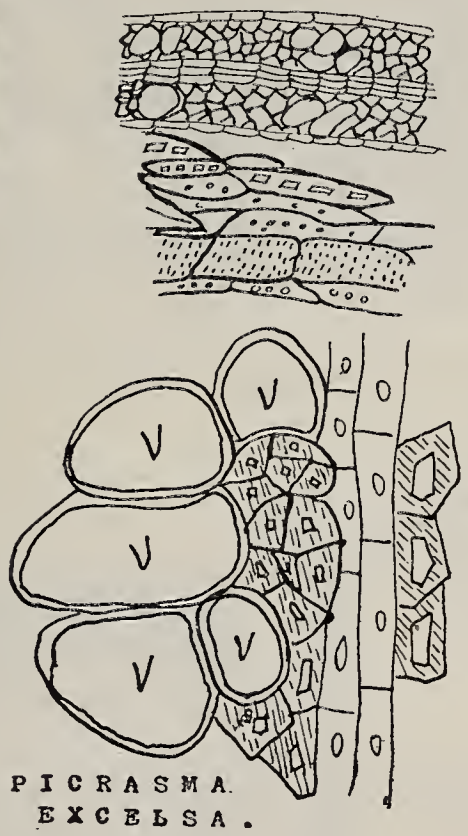
PINE XYLEM.



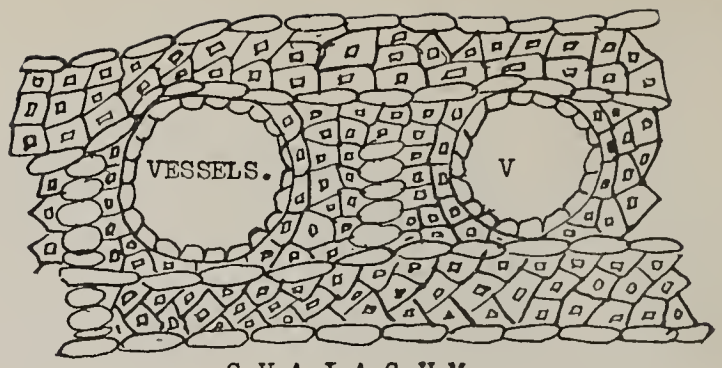
QUASSIA.



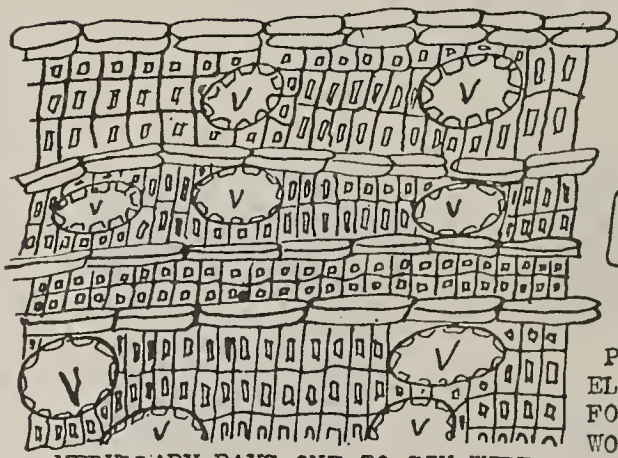
QUASSIA AMARA.



PICRASMA EXCELSA.

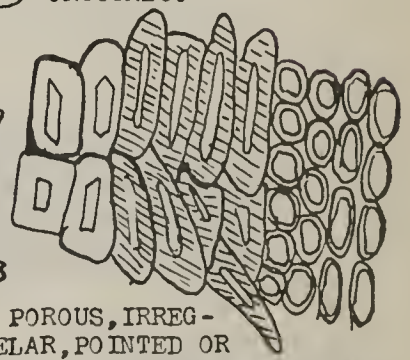


G U A I A C U M .



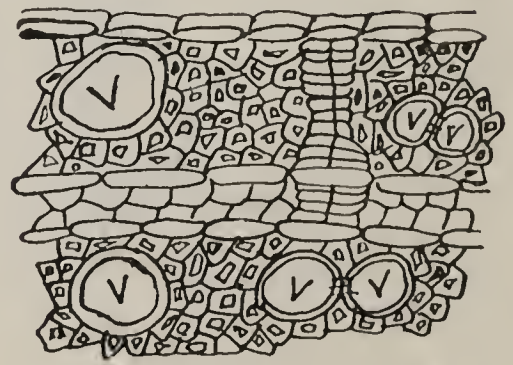
MEDULLARY RAYS ONE TO SIX WIDE

CRYSTALS.



POROUS, IRREGELAR, POINTED OR FORKED ENDS. SOME CRYSTAL WOOD. WOOD AND VESSELS WITH YELLOWISH GRANULAR CONTENT.

S A N T A L R U B R U M .



H A E M A T O X Y L O N

ROOTS

Monocotyledonous, or Endogenous.
  Longitudinally wrinkled, gray brown, thick bark.
Dicotyledonous or Exogenous — SARSAPARILLA.
  With periderm.
    Whole roots.
      Thin bark.
        Reddish, tapering, long, thin, scaly,
          Krameria, Peruvian.
        Reddish, tapering, long, thin, trensversely cells,
          Krameria, Savanilla.
      Thick bark.
        Fleshy fusiform, acrid tingling taste, PYRETHRUM.
        Appearing as a string of beads, IPECACUANHA.
        Cylindrical, twisted and furrowed, tough fracture.
          SCAMMONIAE RADIX.
        Fleshy, keeled and crowned, SENEGA.
    Transverse pieces.
      Thin bark.
        Orange brown, annulate, bitter, Asclepias.
        Yellow, short mealy fracture, collateral F. V. B.,
          Bryonia.
        Concentric zones of collateral F. V. B., Pareira.
      Thick bark.
        Cylindrical, warty, stem scars, Baptisia.
        Yellowish, brown ring, CALUMBA.
        Soft spongy fibrous bark, STILLINGIA.
    Long pieces.
      Thin bark.
        Mealy fracture, odor and taste,
          BELLADONNAE RADIX.
      Thick bark.
        Horny, tough, pith white, Lappa.
        Wrinkled gray bark, yellow wood, resin cells,
          Petroselini Radix.
        Very fibrous, Phytolacca.
        Red brown, dusty fracture, fleshy, Rumex.
Rhizome like.
  Whole.
    With periderm.
      Thick bark.
        Tuber like, cambium 5 to 7 rayed, ACONITUM.
        Tuber like, hard resinous, JALAPA.
    No periderm.
      White gray, fibrous, mucilaginous, ALTHAEA.

ROOTS

The root may be defined as the descending axis of the plant with rootlets or appendages, devoid of leaf forms, or scales, and the growing point back of the apex.

The functions of the root are for support, serving as a foundation, absorption by the unlignified rootlets and hairs, and storage.

All whole roots show epidermal, fundamental and fibro-vascular tissue—the arrangement of these tissues varying, except in the epidermal. Gymnosperms—roots, like the stems, show a very simple structure in the xylem portion—all the cells with the exception of the parenchyma, are replaced by tracheids with bordered pores and show rings of growth and medullary rays. In the monocotyledons, the fibro-vascular bundles, enclosed by an endoderm, are scattered through the fundamental tissue, or there may be only one large bundle as in Sarsaparilla. The dicotyledons have the fibro-vascular tissue arranged radially around a central pith, the xylem and phloem being separated by the cambium zone, crossed by medullary rays, and show rings of growth.

In the monocotyledons, the meristematic tissue soon becomes permanent tissue and the growth practically ceases. The gymnosperms and dicotyledons grow from the meristematic tissue, by constant production of new cells, causing rings of second growth in the xylem. Spiral vessels are not common to this growth. Concentric rings of resin cells characterize some roots as Taraxacum. Latex is principally from the endoderm and mesophloem in the sieve tissue.

The cell contents, such as starch, inulin, gums, oils, resins, mineral matter, et al., are generally found in the fundamental tissue. The phloem is usually rich in rounded cell masses, the xylem in wedges.

Microscopic tests with solutions of iron, phloroglucin and HCI, etc., may separate the cylinders by staining the cell walls so that the xylem and phloem may readily be seen.

Diagnostic Points of Some Roots

Belladonna contains characteristic pitted and pored tracheids, few tracheae, sphenoidal micro-crystal cells, parenchyma with two to six compound starch, few wood cells and no bast fibers (from the stem).

Belladonna has a thick bark, no bast, central wood bundles, surrounded by broad wood wedges and equally wide medullary rays; while Inula has a broad bark, small radially arranged cells, a light circle of cambium. The wood cells

re soft, radially arranged, large vessels in rows. Both the phloem and xylem contain many large resin cells. Inula contains inulin, none in Belladonna, but much starch.

Glycyrrhiza has lines of crystal cells accompanying the bast and wood cells, much starch, calcium oxalate crystals, bast and sieve in groups, no pith in the root.

Ipecacuanha contains single starch, in two, three or four compound, with a central hilum, intermediary fibers or transitional cells, cells with raphides, no true tracheae, but at least three different marked tracheids and no stone cells (from the stem).

Spurious Ipecacuanha has isodiametric parenchyma cells with starch and some raphides in the inner bark. The wood is radiate, and the medullary rays are hard to distinguish from the wood cells. It contains no starch.

Taraxacum contains alternating rings of resin cells, laticiferous and sieve cells. It contains many cells with inulin. This drug is yellow with iodine solutions, differing from Aconitum which is blue. It has no starch. There are non-lignified intermediary fibers. Taraxacum contains laticiferous cells in brown circles, while in Chicory, laticiferous are in radial rows. Both contain inulin.

Sarsaparilla, the only monocotyledonous root official, is characterized by the diagnostic endoderm.

Gelsemium: The parenchyma and collenchyma cells contain a secretion which gives a brown precipitate with Tincture of Iodine, lignified pith. When sections are treated with nitric acid, many of the cells turn a deep yellow, and needle crystals are formed. The wood wedges are separated by medullary rays containing starch, the cork is partly lignified. Some calcium oxalate is present.

Scammoniae Radix has slightly lignified porous stone; the parenchyma contains starch and crystals, many resin ducts; the wood cells are slightly lignified; the cork is thin walled, brownish yellow and lignified.

Althaea: The bast fibers contain starch, very few crystals, but many mucilage cells.

Berberis contains xylem cell walls of yellowish color, many vessels, wood fibres with thick walls, pith cells with starch, clusters of bast and sieve.

Calumba contains sphenoidal micro-crystalline cells, stone cells containing crystals, much starch. Calumba has a thick bark, small wood bundles, broad medullary rays, and much starch in the parenchyma cells; while in Byronia the

bark is thin, small friable wood bundles many rayed and in concentric circles surrounded by thin walled parenchyma.

Krameria contains the diagnostic bast, which is wavy in outline and has attenuated ends and is non-lignified—starch two to four compound—wood cells slightly lignified, and somewhat spindle shaped.

Senega: The xylem turns red brown with KOH solutions. It consists chiefly of tracheids, which resemble wood cells and contain a yellow to red brown amorphous substance. The parenchyma cells of the cortex also contain this substance. This is the drug with a keel.

Senega has irregular yellow porous xylem and irregular cortex. False Senega has a cylindrical whitish xylem and a more regular cortex.

Sanguinaria has many laticiferous and reddish resin cells.

Pyrethrum contains many tracheae with resin, many wood parenchyma cells, but few wood cells; also parenchyma with a yellow brown substance, or Inulin.

Stillingia has many yellow red resin cells and starch, a few calcium oxalate, cork reddish—wood cells soft and porous—thin walled with slit like pores—a few bast which are long and narrow.

Sumbul has yellow brown walled cells, many sieve, laticiferous and bast—wood is irregular and contains resin—the ducts have remnants of end walls.

Pareira has a thin bark, wood in several concentric circles, waxy cut, many uniform porous wood wedges, separated by circles of waxy parenchyma, which resembles medullary rays. The stem has a central pith. The false pareira has very hard wood with concentric circles, not waxy when first cut.

Cimicifuga: The cortex contains collateral fibro-vascular bundles and four to six collateral fibro-vascular bundles in the center, containing many tracheids with acute ends. The rhizomes have five to thirty fibro-vascular bundles.

Veratrum Viride has reddish brown cork, sometimes replacing the epidermis. The fundamental tissue contains starch and crystals, the tracheae contains a lemon yellow substance. The bast are porous.

Veratrum Viride has a cortex of one seventh of its diameter, contains starch, a few crystals, and the wood about one eighth of an inch from the outside—a brown nuclear sheath. The center has many wood bundles, while Cypripedium has a thick cortex, with an indistinct nuclear sheath. The wood

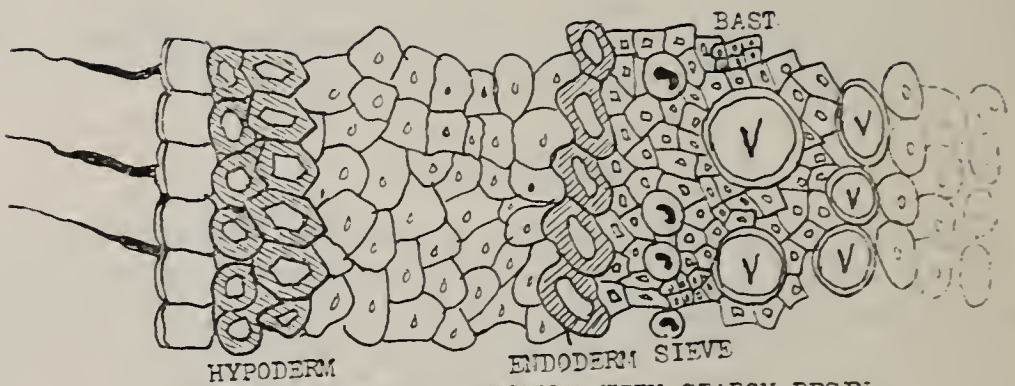
bundles are in the center and are distinct. The parenchyma contains starch.

Hydrastis has sieve cells in clusters near the cambium; many starch grains; the bast fibers are few and very short; cork cells are reddish brown and tabecular.

Valeriana, suberized hypoderm, fundamental tissue contains crystals, oil and starch. The endoderm in the root encloses three to five collateral fibro-vascular bundles. The fibro-vascular bundles of the rhizome are also enclosed by an endoderm, and the pith is larger than that of the root.

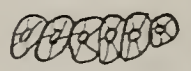
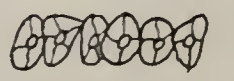
EXERCISE

Outline the various circules visible to the eye, such as the circules about a central pith, then the wood wedges, endoderm or cambium, the cortex and lastly the epidermis. Make Lg. and Tv. sections of Sarsaparilla, Glycyrrhiza. Senega, Belladonna and Apocynum; stain one set of sections with Phloroglucin T. S. and HCI, and another set with C. Z. I.; fill in the various circules with the cells as seen under the low power, then with the high power lens fill in the details. Name each cell. With other sections search for cell contents, using the various reagents for the same. If starch is present note especially the shape, location of hilum and lamella. If crystals are present, note the shapes and kinds. Ask for the powder of the drug you are working on, and by the aid of your U. S. P. name and draw the cells, test for cell contents and note any diagnostic cells or cell contents. Credit will be refused if you use another's text books or material. Hand in at the end of each period a full report. Keep your microscope clean.

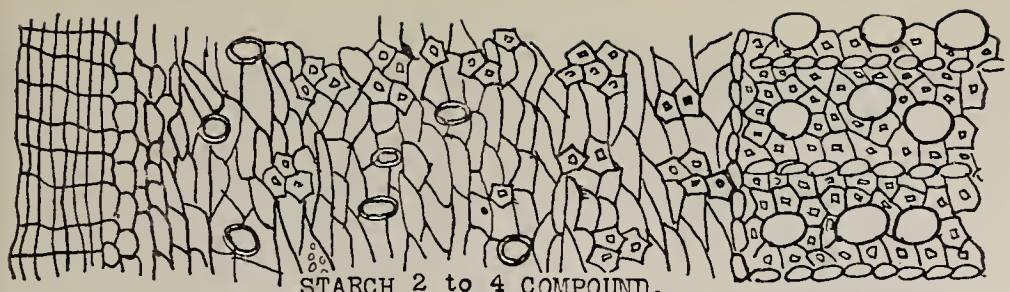


PARENCHYMA AND COLLENCHYMA WITH STARCH, RESIN AND CRYSTALS.

SARSAPARILLA.

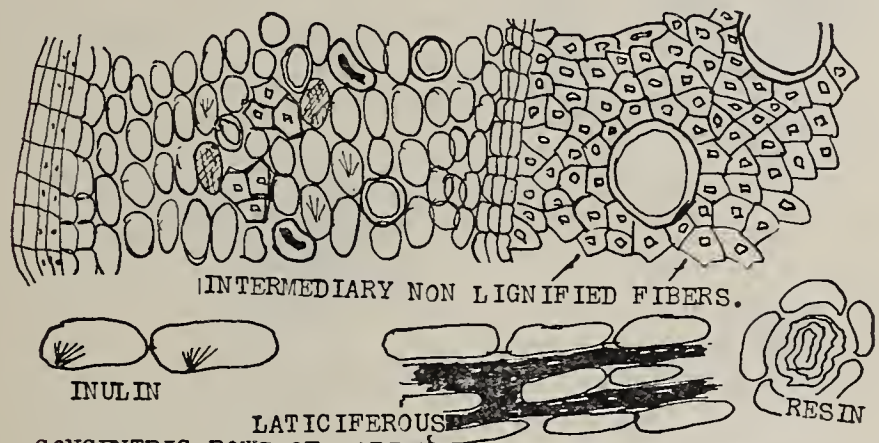
NAME	MEALY OR NON MEALY	STARCH	ENDODERM	WIDTH
HONDURAS	MEALY	MUCH		BARK = WOOD PITH = BARK PITH = WOOD
RIO NEGRA	MEALY	LITTLE		BARK = PITH BARK = 4 TIMES WOOD PITH = 4 TIMES WOOD
MEXICAN	NON MEALY	NONE		PITH = WOOD PITH = BARK WOOD = 2 TIMES BARK
JAMAICA	NON MEALY	NONE		BARK = PITH BARK = I 1/2 TIMES THE WOOD PITH = I 1/2 TIMES THE

VARIETIES OF SARSAPARILLA.



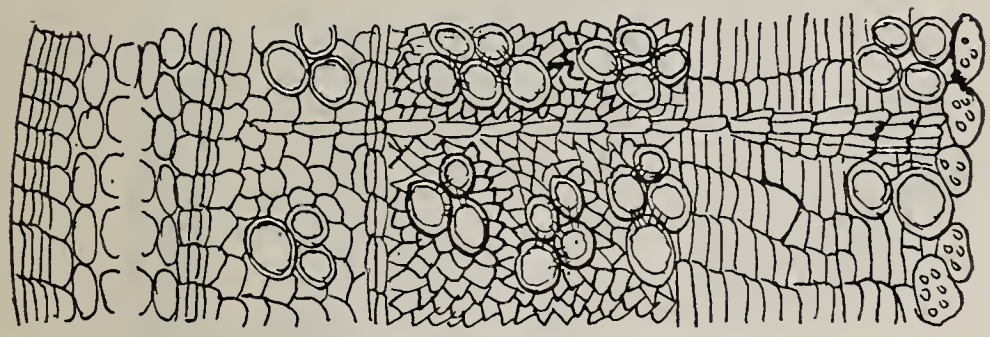
STARCH 2 to 4 COMPOUND.
BAST WAVY,ATTENUATED ENDS,NON LIGNIFIED.
SPINDLE SHAPED,SLIGHTLY LIGNIFIED WOOD.

KRAMERIA.



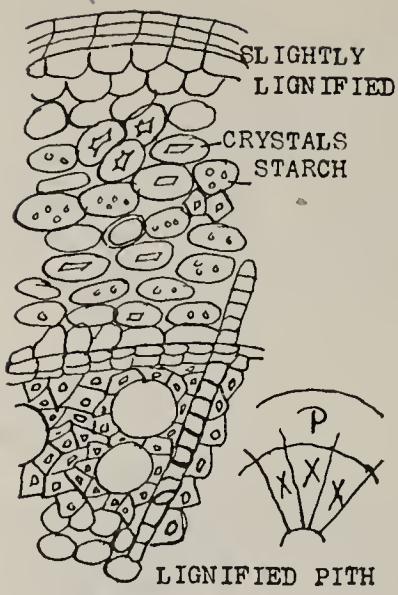
CONCENTRIC ROWS OF LATICIFEROUS, SIEVE AND RESIN
ALTERNATING WITH INULIN BEARING PARENCHYMA.

TARAXACUM.

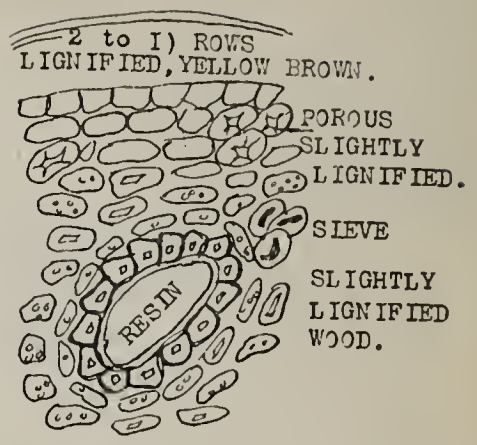


2 to 6 ROWS PARENCHYMA WITH STARCH.FEW WOOD CELLS.MANY
SPHENOIDAL MICRO CRYSTALLINE CELLS. A FEW TRACHEIDS,MANY
DUCTS,MAY BE A FEW STONE. NO BAST (FROM STEM)

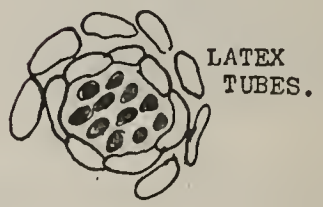
BELLADONNAE RADIX.



GELSEMIUM

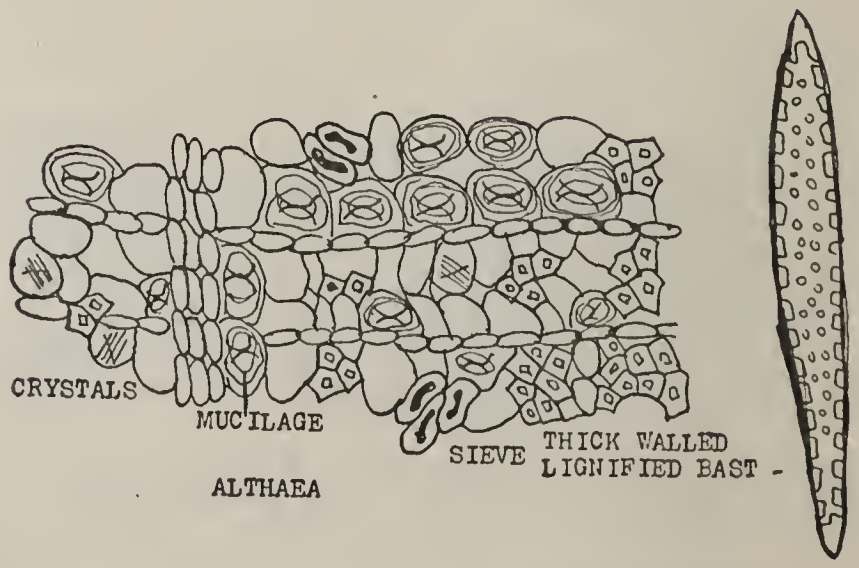


SCAMMONIAE RADIX

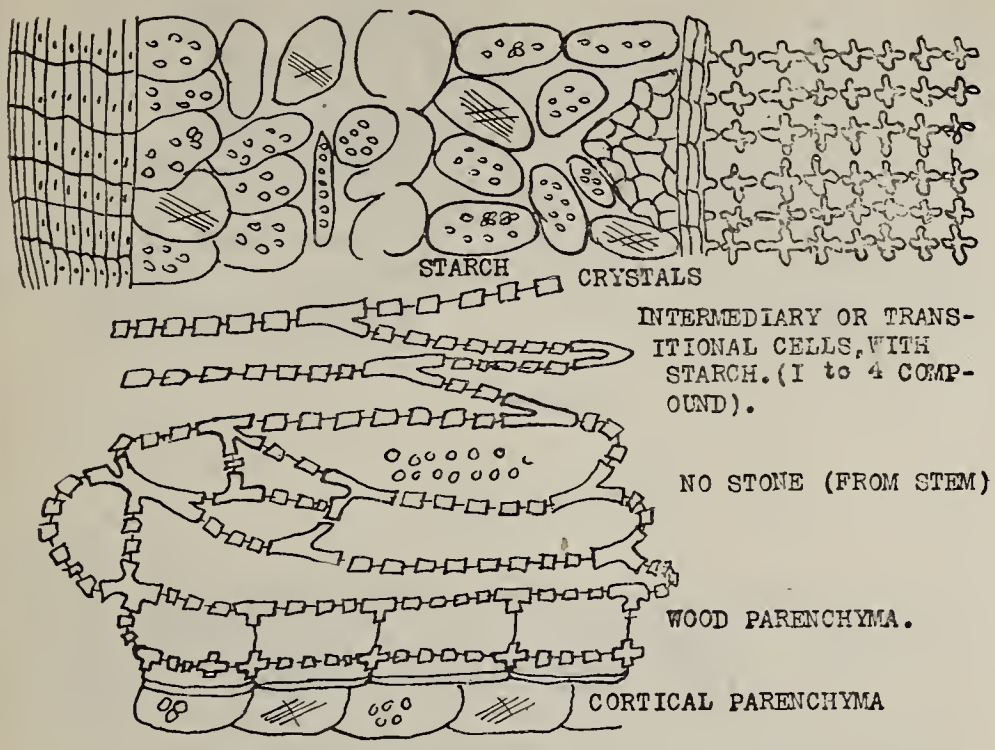


SANGUINARIA

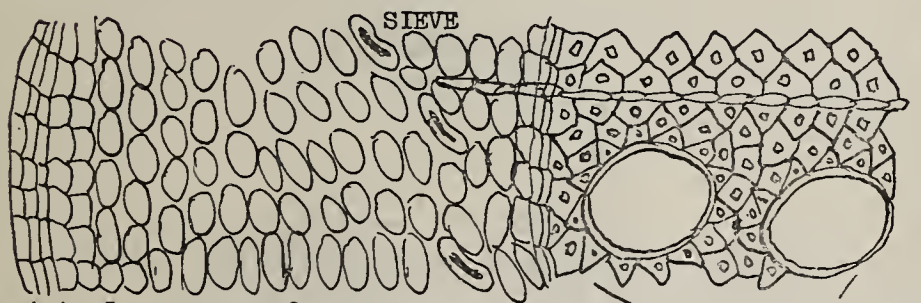
LAPPA



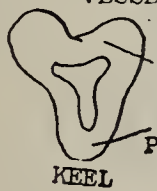
ALTHAEA



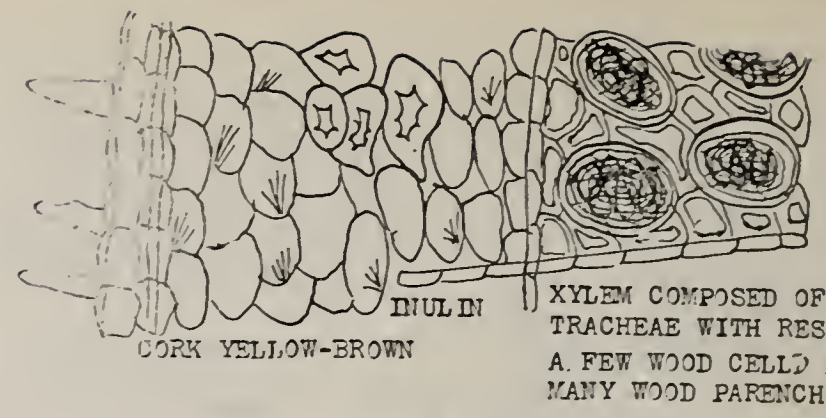
IPECACUANHA.



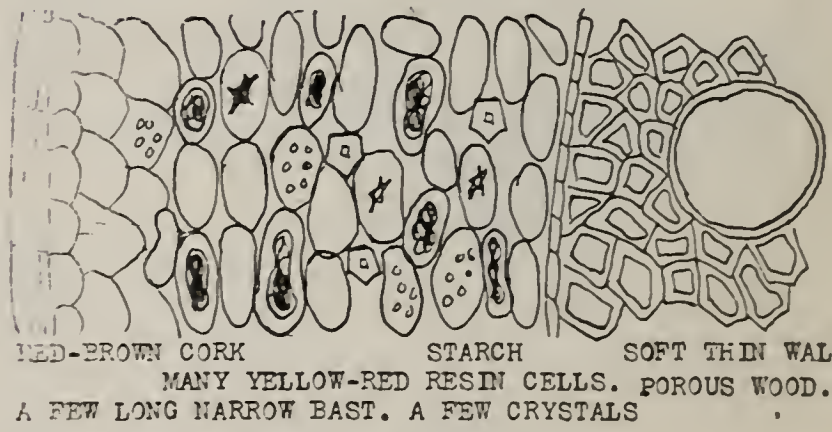
4 to 5 ROWS CORK. 20 CELLS OF PARENCHYMA. IO CELLS WIDE (WOOD) VESSELS MOSTLY TRACHEIDS. MEDULLARY RAYS I to 3 CELLS WIDE AND RESEMBLE SOMEWHAT WOOD CELLS, AND CONTAIN A YELLOW AMORPHOUS SUBSTANCE. XYLEM WITH KOH SOLUTIONS TURNS YELLOW TO BROWNISH RED.



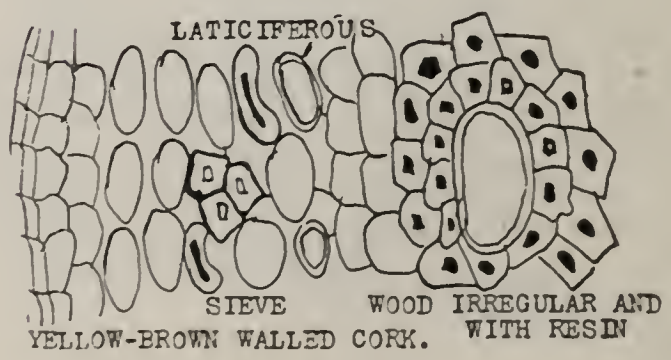
SENEGA.



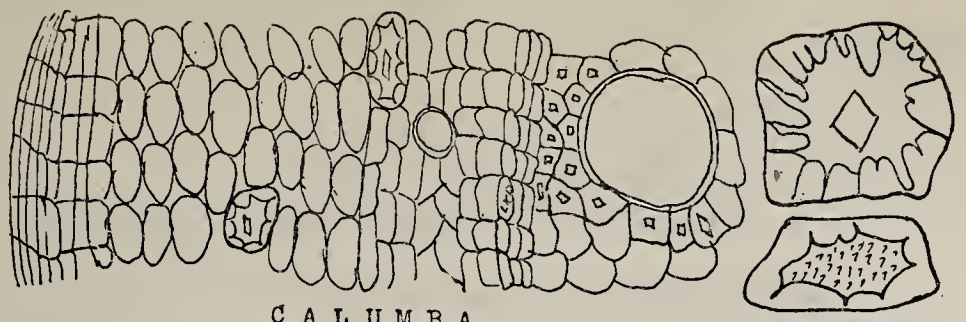
PYRETHRUM



STILLINGIA.



SUMBUL



CALUMBA.

STONE WITH SPHENOIDAL MICRO CRYSTALS

RHIZOMES

Cryptogam or Fern.
  Large, green stype bases, odor on moistening, ASPIDIUM.
Monocotyledon.
  Elongated.
    Horizontal.
      Gray brown, ham shaped, lobed, violet odor. Iris.
    Upright.
      Yellow brown, root scars, odor and taste, Calamus.
  Short.
    With periderm.
      Horizontal.
        Red to gray brown, cut pieces, Zedoaria.
        Red brown, annulate, fibrous, pepper taste, Galangal.
        Yellow brown, stem scars, remnants wrinkled rootlets, Trillium.
        Pale brown, curved, branching, hard, tough, Dioscorea.
    No periderm.
      Gray yellow, lobed, branched, odor and taste, ZINGIBER.
Dicotyledon.
  Short.
    Horizontal.
      Reddish, contorted, tuberculated, Geranium.
      Yellowish, curved, flattened, seal like scars, Scopola.
  Pieces.
    Red brown, white mottling, no periderm, odor, taste, RHEUM.

Monocotyledon.
Horizontal.
Gray brown.
Annulate, light in weight, pithy, Aralia.
Curved, rootlets, wiry, Helonias.
Many soft rootlets, annulate, tufts of leaf bases, Aletris
Wrinkled, flattened, annulate, Iris versicolor.
Orange brown, wavy rootlets, stem scars, Cypripedium.
Straw color.
Hair like rootlets, Convallariae Radix.
Resembles transverse cut pieces of hay, Triticum.
Upright.
Black gray, shriveled annulate rootlets, VERATRUM VIRIDE.

Dicotyledon.
Horizontal.
Dark brown.
Branched, flattened, long black rootlets, LEPTANDRA.
Seal like scars, white root scars, PODOPHYLLUM.
Gray brown.
Small, knotty, stem scars, long rootlets, SPIGELIA.
Thick, rounded, long rootlets, Inula.
Longitudinally wrinkled, twisted, odor, taste, GENTIANA.
Purple brown.
Cylindrical, twisted annulate, wood with light and dark wedges, Echinacea.
Thin, contorted, interior whitish, Asarum.
Brownish to blackish in cut pieces, large light wood, bark separates easily, Kava.
Yellow, small, knotty, brittle yellow rootlets, HYDRASTIS.
Yellow brown.
Thin, greenish bark, yellow radiate wood, Beberis.
Twisted, fissured, porous radiate wood, Gelsemium.
Fibrous, roughly longitudinally wrinkled, sweet taste, GLYCYRRHIZA, Spanish.
Yellow green, no cortex, fibrous, sweet, GLYCYRRHIZA, Russian.
Gray, resin cells in porous yellow wood, Angelicae Radix.
Reddish, hard, irregular, many resin cells, SANGUINARIA.

Oblique.
Brownish, wiry rootlets, turpentine odor,
SERPENTARIA.
Upright.
Red brown, fusiform, yellow wood, resinous,
TARAXACUM.
Pale brown, scaly bark, white wood, Hydrangea.
Yellow brown.
Annulate above, lower surface warty, large,
Pimpinella.
Hard, irregular, bent, stem scars, rootlets,
Caulophyllum.
Brownish black, large, irregular, knotty, many rootlets,
CIMICIFUGA.
Dark brown, many rootlets, odor, VALERIANA.

TUBERS

Whole.
Yellow brown, hard, rounded, triangular scar, opaque,
Corydalis.
Transverse pieces.
Grayish, reinform, COLCHICI CORMUS.

BULBS

Pieces.
Pinkish white, hard, curved, SCILLA.
Gray white, odor, Allium.

RHIZOMES

Rhizomes are stems which resemble roots, growing partly or wholly under ground, but unlike roots, they show leaf scars, or some modifications. Many rhizomes show cup shaped scars and rootlets, usually at the nodes.

Rhizomes have practically the same structure as roots, except in some cases the pith is larger.

They are described according to the manner of growth, which is shown by the position of the leaf and root scars as "horizontal", "oblique", and "vertical". The form and shape range from the stem like or bulbous as Scilla, to that of Colchici Cormus—Scilla with short nodes and leafy scales—Colchicum Cormus with thick internodes and membranous scales.

Rheum, Aconitum (tuberous), Jalapa (tuberous), Gelsemium, Berberis and Gentiana are true rhizomes, but are frequently described as roots, which they closely resemble.

One fern rhizome—Aspidium, one corm—Colchici Cormus, one bulb—Scilla and two tubers—Aconitum and Jalapa, are official.

Where leaf modifications occur, the characteristic tissue of leaves may be found. Rootlets have practically the same microscopical structure as roots, but usually rudimentary.

Parenchyma predominates in the fleshy forms; sclerenchyma in the hard fibrous forms. Starch is the most common cell content.

Generally the dicotyledon roots and rhizomes show no scars or leaf remnants.

Microscopically, three types are studied:

1. In the monocotyledons, the stem and roots are much alike, showing a sheath or endoderm, near which the fibro-vascular bundles are found on either side; the type of bundle is usually closed collateral or closed concentric.

2. In the dicotyledons, the rhizomes resemble the stem, showing a central pith, woody cylinder arranged in wedges, medullary rays, cambium zone separating the xylem and phloem. The phloem is external to the xylem, with one exception as in Rheum. A mass of fundamental tissue—cortical surrounds the cylinders described, and it is surrounded by a hypoderm or its corky representative, in some rhizomes.

3. Vascular cryptogams are each one peculiar to itself, as Aspidium.

Diagnostic Points of Some Rhizomes

Rheum with its xylem exterior to the phloem, contains rosette crystals of calcium oxalate about one third its own weight. Sections turn red with alkalies and show two to four compound starch grains—few tracheae—medullary rays irregular.

Gentiana is diagnosed by the absence of elements, for there are no bast, stone, wood, starch or tannin, only a few crystals, many wood parenchyma cells. When solutions of potassium hydroxide are added to the sections, a red color should immediately show; if the red color is absent, the drug is inferior, or has been exhausted.

Jalapa contains pear shaped fibro-vascular bundles, many laticiferous, starch one to three compound, with a pear shaped split hilum, similar to potato, some altered starch, spiny rosette crystals, many yellow brown resin cells.

Pipstostegia pisonis—Brazilian jalap occurs in transverse or oval sections, marked with concentric rings, and of

a pale yellowish color, and with many translucent pale resin dots.

Colchici Cormus contains one to two up to six compound starch, cubical, triangular or star shaped hilum.

Zingiber has many oleoresin cells; if the ginger is fresh these cells are filled with oil and the walls are yellow; but if old, the walls are brownish and contain a hard resin, which is insoluble in alcohol, ether or chloral solution, acetic acid, or alkalies. The starch is diagnostic, the hilum is excentric. The fibro-vascular bundles are closed. If cork is present, the rhizome has not been peeled. In the confection, the secretion cells have an oily content of a yellow color.

Triticum has lignified hypoderm cells, collateral fibro-vascular bundles, few starch grains, parenchyma with a carbohydrate, and lignified epidermal cells.

Apocynum contains many laticiferous cells, and is a typical dicotyledon rhizome, with the exception of stone cells, which are found in the Androsaemifolium.

Aconitum contains diagnostic starch, one to two to four compound, hilum centric, stone cells, and irregular wood cells, no bast (from the stem) star shaped cambium enclosing five to twelve fibro-vascular bundles, many tracheae with slit like pores.

Podophyllum has twenty-four to thirty-six collateral fibro-vascular bundles, groups of sieve and bast, starch two to six compound, resin cells, crystals, more crystals in the nodes.

Aspidium, the only fern rhizome official, contains six to twelve fibro-vascular bundles enclosed by an endoderm, oil or large resin cells and diagnostic starch.

Calamus contains large intercellular spaces, parenchyma with starch, many resin cells, fibro-vascular bundles, enclosed by an endoderm.

Sanguinaria has sphenoidal micro-crystals, collateral fibro-vascular bundles, rows of resin cells. The starch resembles that of wheat.

Scilla contains characteristic needle sacs, full of acicular crystals, mucilage cells.

Diagnostic Points of Some Rhizomes and Roots

Spigelia has heavy walled parenchyma, no stone, a few bast, which are long and non-lignified. Sieve resembles parenchyma. Few ducts, which resemble tracheids. The root contains six to eight rounded fibro-vascular bundles.

Convallaria shows hypoderm, parenchyma and collenchyma with starch and crystals—Pith of rhizome with large

intercellular spaces. The root contains more oil cells and raphides than the rhizome.

Leptandra: Sections turn pink with chloral test solution. It has starch and many resin cells. The wood cells resemble tracheids. It has hypoderm cells.

Cypripedium contains starch and crystals, thick walled wood fibers, endoderm.

Serpentaria: The stem contains six to ten fibro-vascular bundles, the root four to six rayed, and the rhizome six to ten; also parenchyma with chloroplasts, starch and oil, bast single or in circles, lignified medullary cells, laticiferous in groups near the cambium—large pith.

Some of the Rhizomes and Roots Containing Crystals:

Raphides—Phytolacca, Ipecacuanha, Sarsaparilla, Veratrum.

Sphenoidal micro-crystals—Belladonna.

Prisms—Calumba, Stillingia, Krameria, Glycyrrhiza, Scammonia.

Rosettes—Jalapa, Althaea, Rheum.

No crystals—Senega, Lappa, Chicory.

No bast—Gentiana, Spigelia, Ipecacuanha, Taraxacum and Belladonna.

Stone cells—Aconitum, Calumba.

No stone cells—Chicory, Taraxacum, Lappa, Pyrethrum, Gentiana, Senega.

Inulin—Lappa, Pyrethrum, Taraxacum, Chicory.

Characteristic starch—Aconitum, Althaea, Belladonna, Calamus, Ipecacuanha, Jalapa, Krameria, Sarsaparilla, Stillingia.

No starch—Gentiana, Senega.

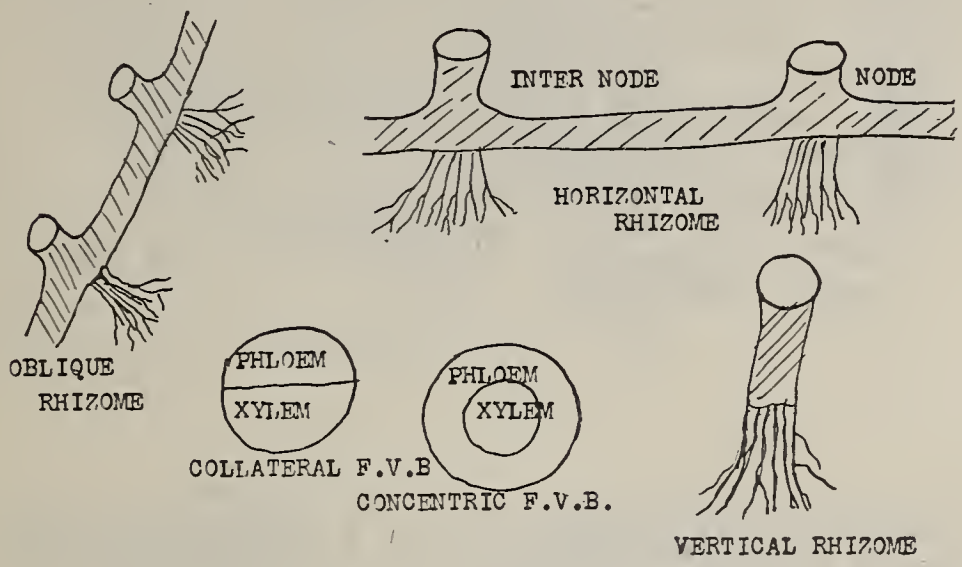
Tracheae—Aconitum, Angelica, Apocynum, Arnica, Asclepias, Chicory, Jalapa, Inula, Petroselinum, Pimpinella, Pyrethrum, Serpentaria, Stillingia, Sumbul, Taraxacum, Valeriana.

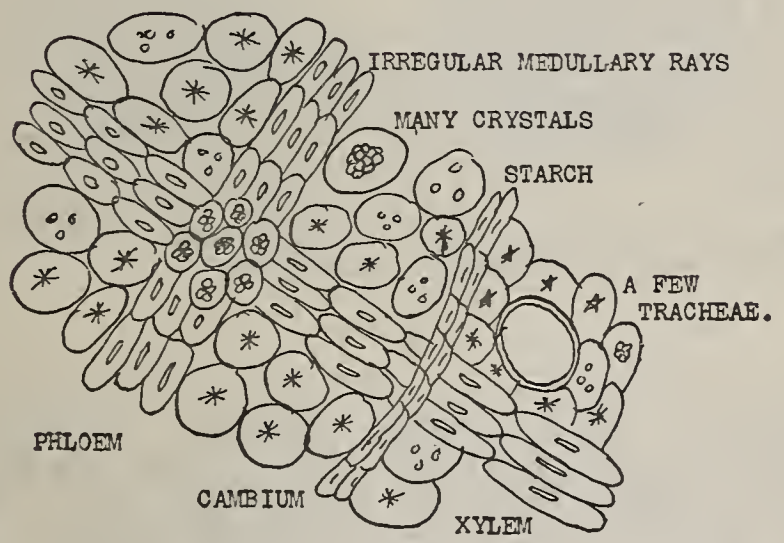
Few or no tracheae—Althaea, Belladonna, Bryonia, Calumba, Gentiana, Ipecacuanha, Lappa, Leptandra, Phytolacca, Rheum, Senega, Spigelia.

EXERCISE

Make Lg. and Tv. sections of Aconite, Ginger, Gentian and Aspidium; if rootlets are present, these also must be sectioned; if leaf forms, make Tv. and Epidermal sections. Draw under the low power and fill in details with the high power lens. Name and draw all the cells and cell contents, giving the stains and reagents used and their results. Note

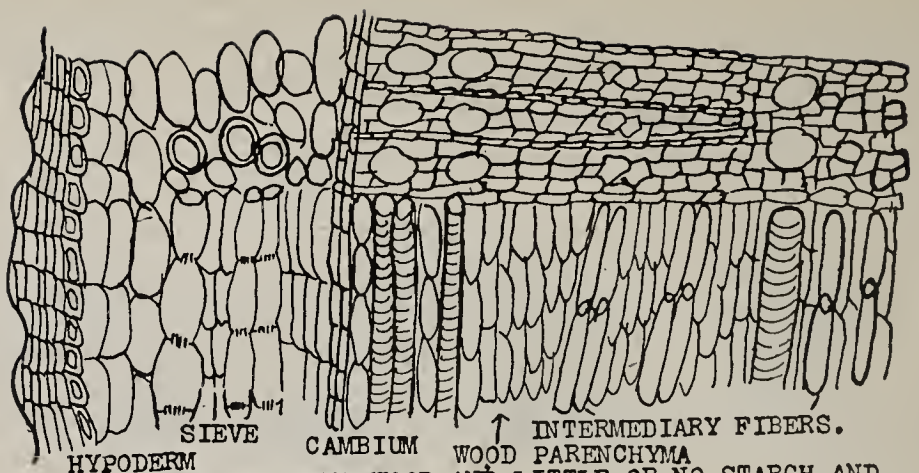
the starch and crystals if present, also the character of the vessels and reservoirs, bast and stone. Then ask for the powdered drug and report as outlined under roots. Bring your U. S. P. each day.





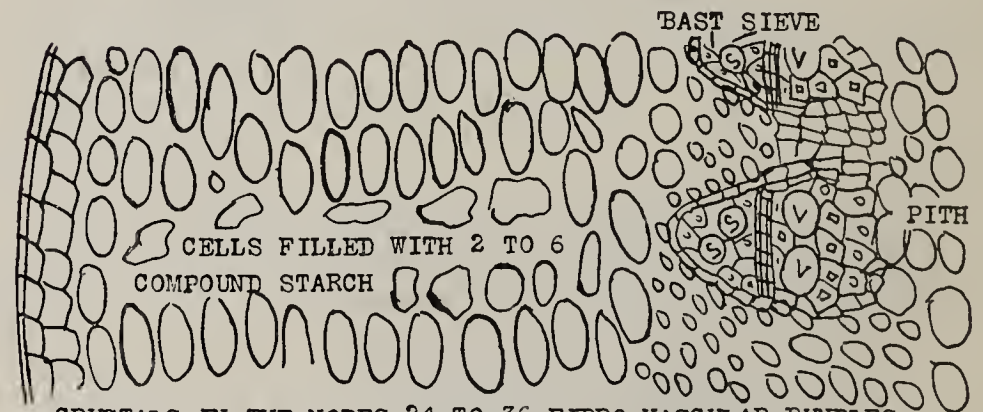
STARCH 2 to 4 COMPOUND. XYLEM EXTERIOR TO PHLOEM. ABOUT I/3 THE WEIGHT IS CALCIUM OXALATE. SECTIONS TURN RED WITH ALKALIES.

RHEUM



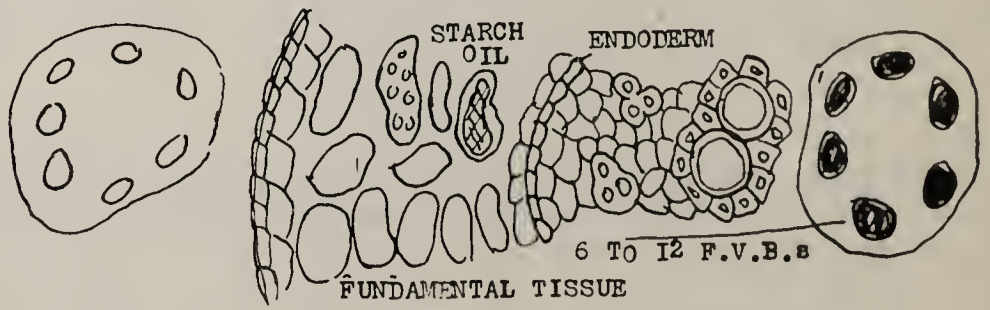
NO BAST, STONE, WOOD AND LITTLE OR NO STARCH AND TANNIN. A FEW CRYSTALS.

G E N T I A N A .

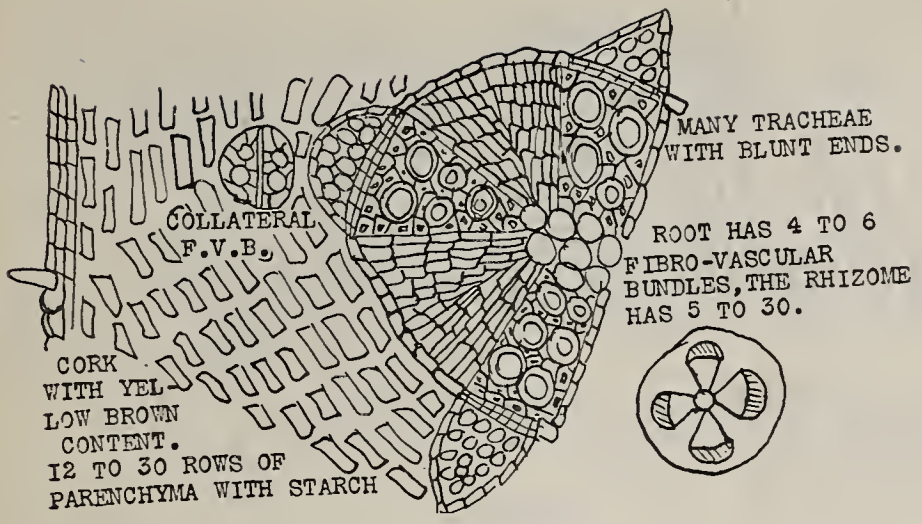


CRYSTALS IN THE NODES. 24 TO 36 FIBRO-VASCULAR BUNDLES, SEPARATED BY WOOD PARENCHYMA AND PARENCHYMA

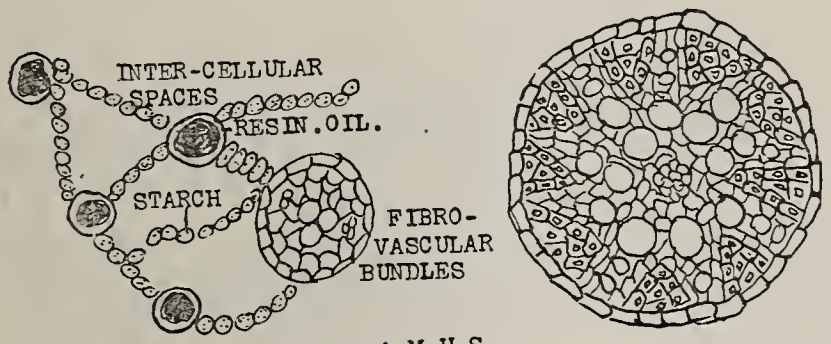
P O D O P H Y L L U M .



A S P I D I U M .



C I M I C I F U G A .



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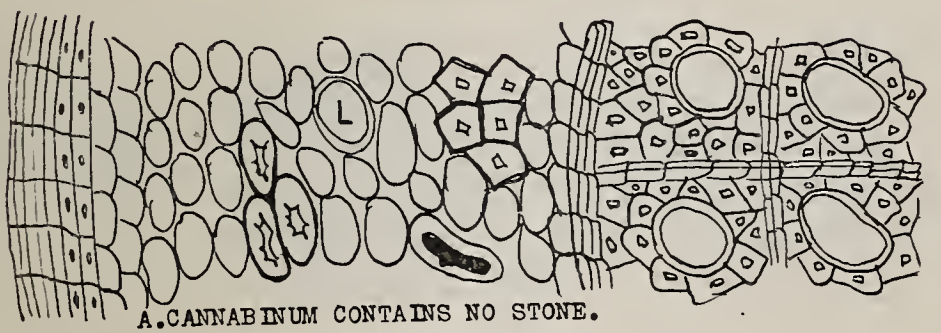
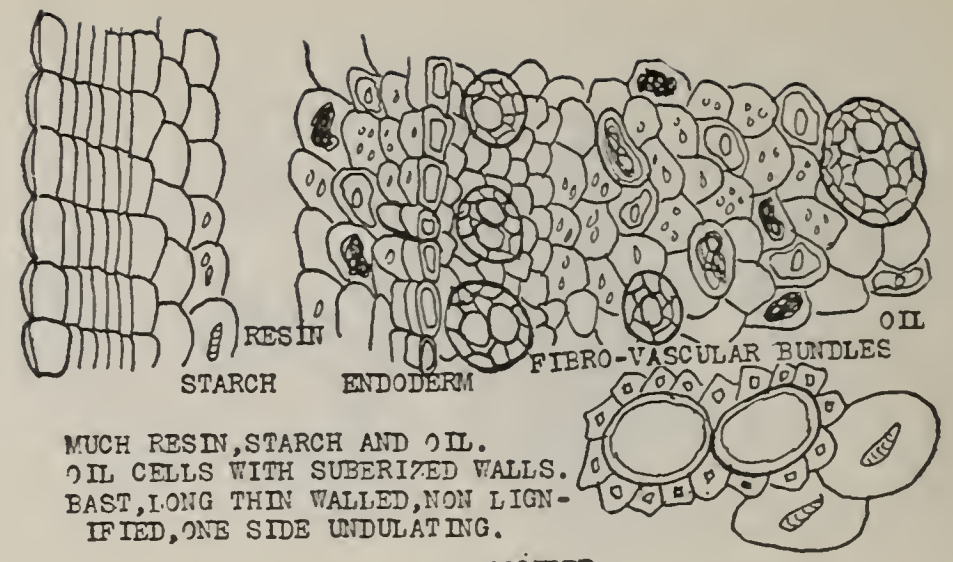


A P O C Y N U M .

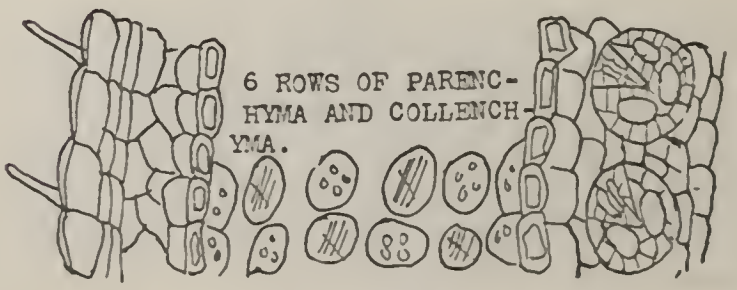


MUCH RESIN, STARCH AND OIL.
OIL CELLS WITH SUBERIZED WALLS.
BAST, LONG THIN WALLED, NON LIGNIFIED, ONE SIDE UNDULATING.

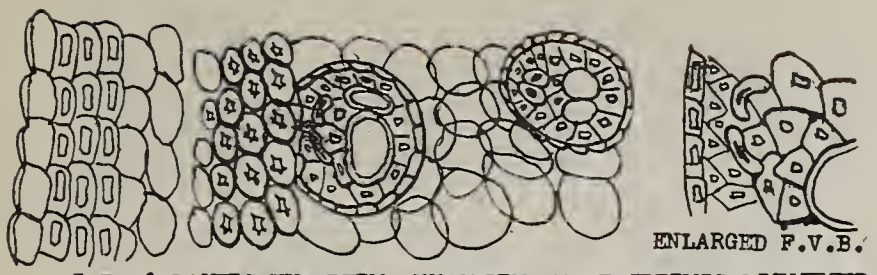
ZINGIBER.

IN THE FIBRO-VASCULAR BUNDLE, THE XYLEM IS V SHAPED, THE SIEVE IN THE UPPER PART OF THE V.

CONVALLARIA (RHIZOME)

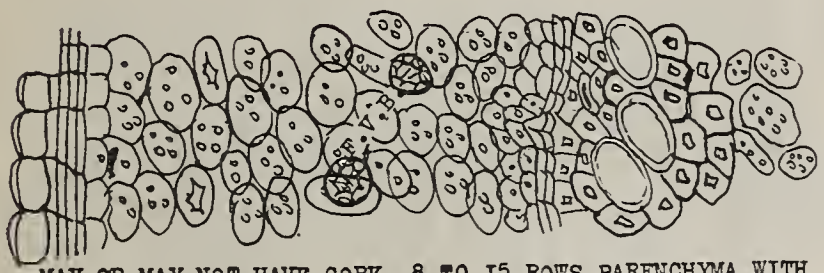


CONVALLARIA (RADIX)



3 TO 6 LAYERS HYPODERM. HYPODERM AND EPIDERMIS LIGNIFIED. IO TO I6 LAYERS PARENCHYMA, CONTAINING SOME STARCH AND A CARBOHYDRATE. CONTINNOUS LAYER OF STONE CELLS. 8 TO IO LAYERS PARENCHYMA. ENDODERM ENCLOSING THE COLLATERAL FIBRO-VASCULAR BUNDLES, WHICH ARE COMPOSED OF BAST, SIEVE, TRACHEAE AND WOOD FIBERS.

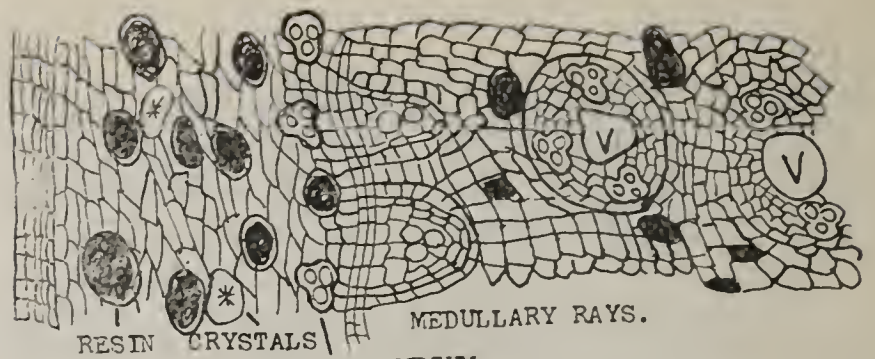
T R I T I C U M .



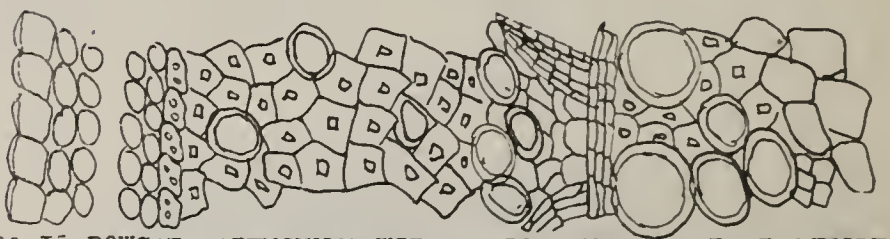
MAY OR MAY NOT HAVE CORK. 8 TO I5 ROWS PARENCHYMA WITH STARCH, STONE CELLS SINGLE OR GROUPED, NO BAST (STEM) CAMBIUM WITH STARCH. FIBRO-VASCULAR BUNDLES, COLLATERAL, WOOD FIBERS IRREGULAR IN OUTLINE.

A C O N I T U M .

H Y D R A S T I S .

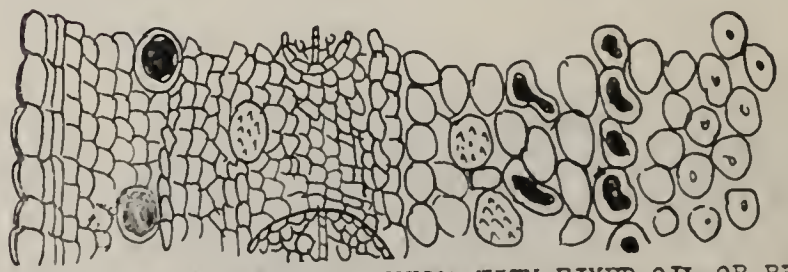


J A L A P A .



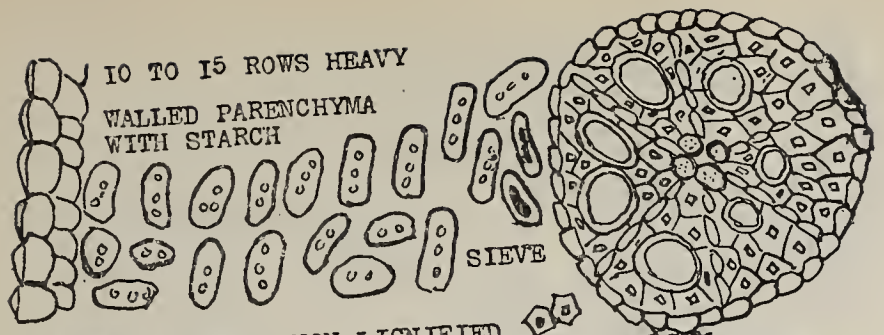
TO 15 ROWSOF PARENCHYMA WITH STARCH AND OIL. BAST SINGLE OR IN CIRCLE. LATICIFEROUS GROUPED NEAR THE MEDULLARY RAYS AND CAMBIUM. THE MEDULLARY RAYS ARE ABOUT 8 CELLS WIDE. THE ROOT IS 4 TO 6 RAYED AND THE LIGNIFIED PITH IS CENTRIC. THE RHIZOME PITH IS EXCENTRIC. THE STEM CONTAINS 6 to 10 FIBRO-VASCULAR BUNDLES.

S E R P E N T A R I A .



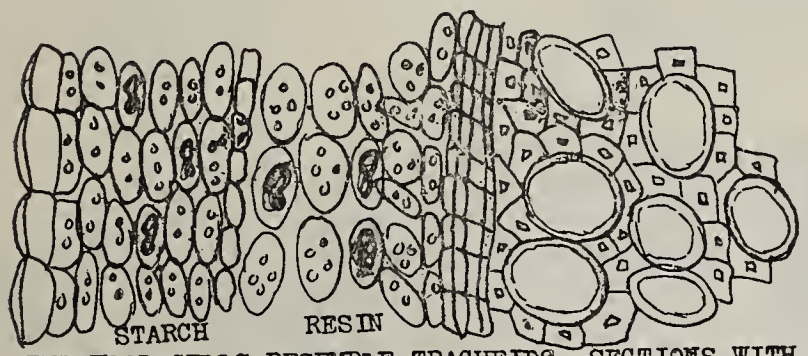
10 TO 15 ROWS PARENCHYMA WITH FIXED OIL OR RESIN AND STARCH RESEMBLING THAT OF WHEAT. MANY LATEX CELLS, SCATTERED SPHENOIDAL MICRO CRYSTAL CELLS.

S A N G U I N A R I A .



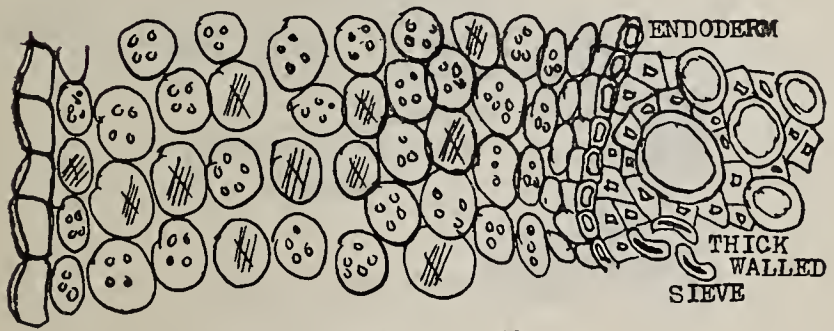
A FEW BAST,LONG, NON LIGNIFIED
A FEW TRACHEAE,BOTH TRACHEAE AND
TRACHEIDS ARE PORED.NO STONE. SOME PARENCHYMA RESEMBLES SIEVE. STARCH IN THE PITH. THE ROOT CONTAINS 6 TO 8 RADIAL FIBRO-VASCULAR BUNDLES.

S P I G E L I A . (RHIZOME)

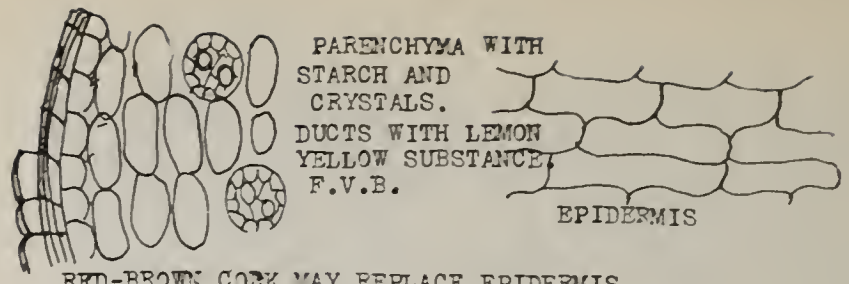


THE WOOD CELLS RESEMBLE TRACHEIDS. SECTIONS WITH CHLORAL T.S. TURN PINK.

L E P T A N D R A

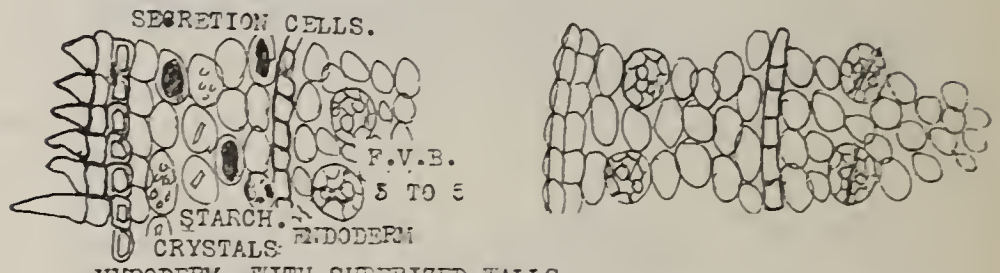


C Y P R I P E D I U M .



RED-BROWN CORK MAY REPLACE EPIDERMIS.

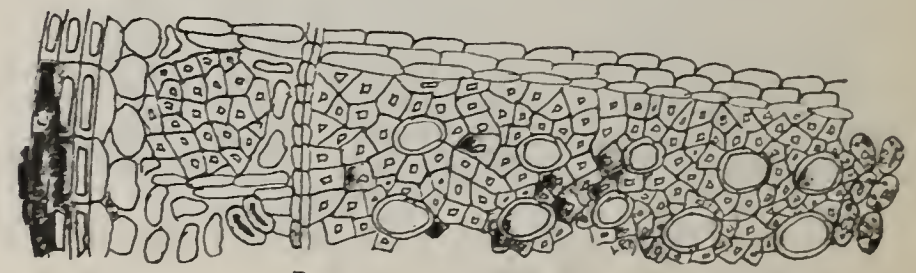
VERATRUM VIRIDE



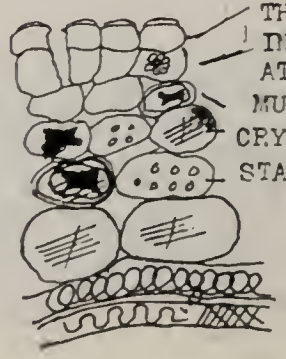
HYPODERM WITH SUBERIZED WALLS.

VALERIANA (ROOT)

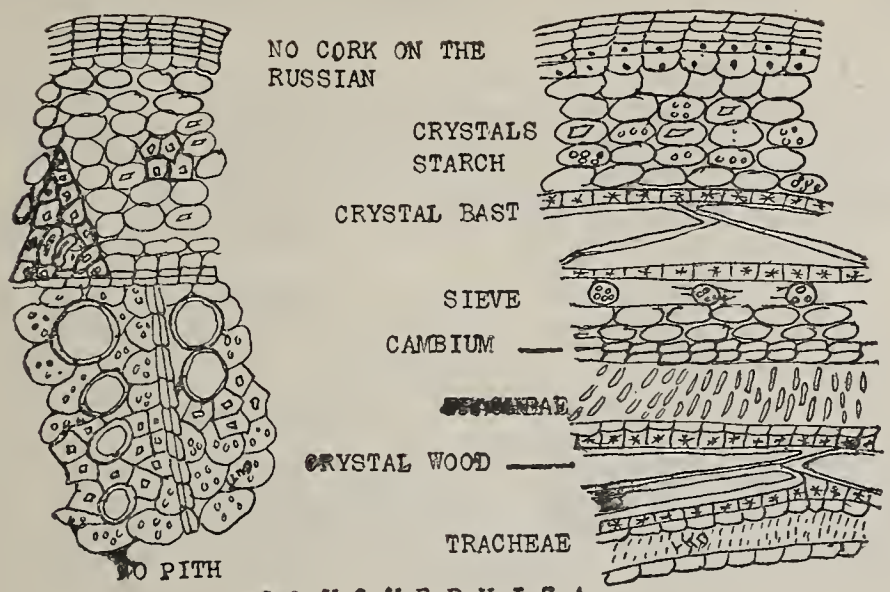
VALERIANA (RHIZOME)



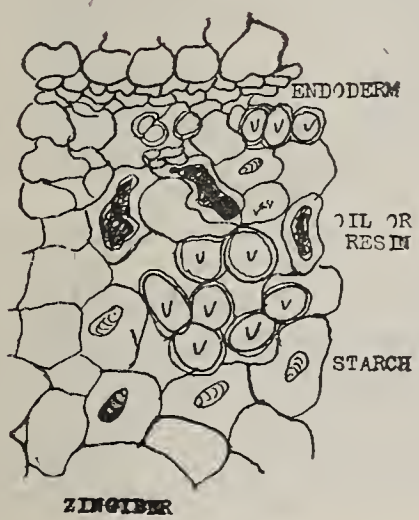
B E R B E R I S .



S C I L L A .



G L Y C Y R R H I Z A.—



ZINGIBER

STEMS

The stem of the plant is distinguished from the root by having leaves or leaf traces, and the growing point at the apex. Rhizomes, tubers, bulbs and corms are underground stems.

Three types of stems are recognized: Vascular cryptogams, Monocotyledons and Dicotyledons. The monocotyledons are more regular and uniform in structure than the cryptogams. The stem and root are closely alike in structure, while the dicotyledons are alike in structure and are the most uniform of all.

1. The cryptogams show an epidermis, which may be replaced by cork, five to eight layers of corky tissue in some stems or replaced by a hypoderm. The fundamental tissue is usually rich in starch and contains many oil cells. The starch is the most characteristic cell content. The endoderm surrounds the closed fibro-vascular bundles. The xylem contains very small tracheae and tracheids. In some cases the structure and resin cells are diagnostic, as in Aspidium.

2. The monocotyledons show a thin endoderm, an inner cylinder containing the closed concentric fibro-vascular bundles. The fundamental tissue consists of rings of parenchyma, about the large intercellular spaces. These rings may be broken by large oil or resin cells. Starch and tannin are a common cell content and are easily detected, and sometimes must be removed before the structure can be clearly seen and studied.

3. The dicotyledons show a central woody cylinder with a parenchymatous pith. The xylem is separated from the phloem by the cambium zone. Distinct medullary rays, epidermis and cortex are seen.

In general the elements are epidermal cells, (may be lignified), cork, stone, parenchyma and collenchyma, crystal, mucilage oil or resin and tannin cells, and any cell of the xylem or phloem may be present. The hypoderm is usually composed of thick walled oblong cells, which may be cutinized or lignified.

The adulterants are chiefly accidental, especially in the powdered drug, such as petioles, leaves and root tissue.

EXERCISE

Make longitudinal and transverse sections. Study as for rhizomes. Make full and complete reports. Use Dulcamara and Chirata as specimens.

Structures of Stems

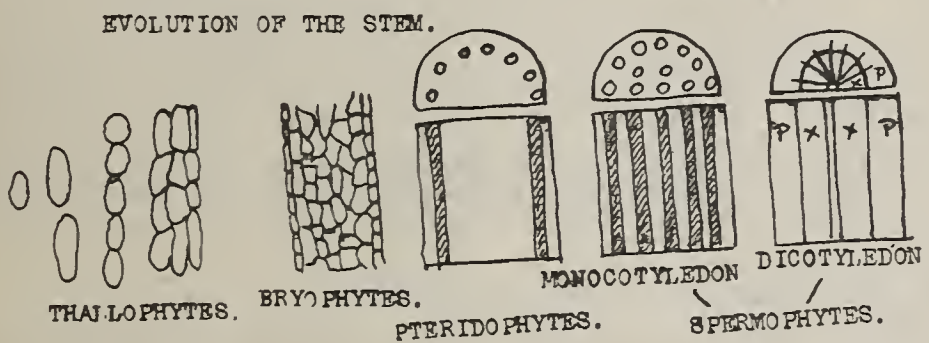
Primary structure: Grows from the meristematic layer : the apex.

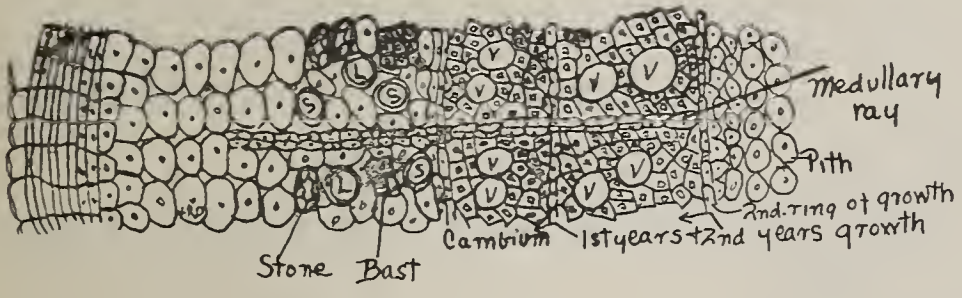
1. Epidermis with trichomes.
2. Hypoderm.
3. Cortex.
4. Epidermis.
5. Collateral bundles, separated by primary medullary ays.

Secondary structure: Dicotyledons and a few gymnoperms.

1. Primary structure.
2. Primary cortex.
3. Epidermis.
4. A few open collateral bundles, pith.
5. Secondary cortex.

Open collateral bundles may be separated by secondary undles. Medullary rays and pith cells are frequently disntegrated. They contain annular rings of growth—complete hloem with sometimes the epidermis replaced by cork.





TYPICAL STEM (DULCAMARA)

Whole.
Coriaceous.
Entire margin.
Glandular punctate.
Gray to yellow green, 5 to 11 leaflets.
Oblong, 15 cm. by 4.5 cm., PILOCARPUS, Jaborandi.
Ovate oblong, 5 cm. by 2.2 cm., PILOCARPUS, Microphyllus.
Scythe shaped, gray green, not hairy, odor, EUCALYPTUS.
Not glandular punctate.
Brown green, oval, UVA URSI.
Margin not entire.
Glandular punctate.
Yellow green, odor and taste.
Oval, less than 26 mm. by 21 mm., BUCHU, Short.
Linear, about 4 cm. by 10 mm., BUCHU, Long.
Gray green, lanceolate, 8 to 10 leaflets, taste.
Pinnately compound, 5.5 cm. by 16 mm., SENNA, Indian.
Pinnately compound, 4.2 cm. by 17 mm., SENNA, Alexandria.
Light green, obovate, 4 to 12 dentations, hairy, Damiana.
Dark green, oblanceolate, Chimphilla.
Brown green, oval, rough surfaces, lower hairy, Boldo.
Not coriaceous.
Margin entire.
Green brown, obovate oblong, raised line near edge, Coca, N. O.
Margin not entire.
Gray green, obovate oblong, very hairy, odor, Salvia, N. O.
Crumpled or broken.
Coriaceous.
Margin not entire.
Yellow gray green, lanceolate, serrate, resinous, hairy, ERIODICTYON.
Not coriaceous.
Margin entire.
Ash green, obovate, sessile, glabrous, odor, Menyanthes.
Margin not entire.

Gray green.
Obovate, lower surface reticulate, hairy, DIGITALIS.
Ovate, sinuate, lobed, wrinkled, STRAMONIUM.
Oval lanceolate, crenate, hairy, Verbasci Folia.
Reniform, crenate dentate, palmate, hairy, Altheae Folia.
Orbicular, lobed, dentate, hairy, Farfara.
Yellow green, cordate, serrate dentate, thin, hairy, Malvae Folia.
Brown green, lanceolate, crenate, lower surface reticulate, Matico.
Pale brown green.
Oblong lanceolate, serrate, feather veined, Castanea.
Obovate, sinuate, brittle, short petiolate, large, Hamamelidis Folia.

LEAVES AND FLOWERING TOPS

Ovate.
Lobed, hairy, sinuate, yellow or red flowers, HYOSCYAMUS.
Serrate, purple stems, odor, taste, MENTHA PIPERITA.
Serrate, green stems, odor, taste, MENTHA VIRIDIS.
Sessile, odor, axillary flowers. Thymus.
Alternate, pubescent, pale blue raceme flowers, Lobelia.
Ash green, axillary flowers, BELLADONNAE FOLIA.
Lanceolate, cuneate, perfoliate, flowers white, Eupatorium.
Pinnate, pubescent, flowers yellow, Absinthium.
Spatulate, sessile spinosed, flowers yellow, GRINDELIA.
Cordate, crenate, downy, flowers white, purple dotted, Cataria.

FLOWERING PLANTS

Small, reddish, rootlets black, flowers white, Drosera.
Green oval leaves, raceme flowers, Centaurum.
6 to 8 pairs ovate lanceolate leaflets, raceme, flowers white to violet, Galega.
1 to 3 oval oblong leaflets, racemes yellow, Melilotus.

HERBS OR PLANTS

Red roots, dichotomous stem, oblong leaves, small flowers, Euphorbia Pilulifera.
Pubescent hollow stems, orbicular leaves, tendrils, yellow flowers, Passiflora.
Obovate, 3 lobed leaves, flowers white, thread like yellow rhizome, Coptis.

Oblong, revolute, branched stems, red yellow flowers, Helianthemum.
Oblong lanceolate, purple green leaves, spikes, blue or purple flowers, Verbena.
Ovate, oblong, serrate leaves, flowers pale blue, Scutellaria.
Linear, pinnately cleft, large purple bell flowers, Pulsatilla.
In bundles, ovate sessile leaves, small flowers, 4 sided roots, Chirata.
Pinnate divided, linear, hairy gray stems, flowers yellow, Adonis.
Rounded crenate leaves, yellow corymbs, Senecio.

STEMS AND BRANCHES

Cylindrical, hollow, warty, greenish, Dulcamara.
5 to 7 angled spinous fresh stems preserved in alcohol, Cactus Grandiflorus.
Green to red brown, fibrous 5 angled, Scoparius.
Green brown, turpentine odor, Thuja.

FLOWERS

Creamy color, campanulate, 5 lobed, odor, Sambucus.

FLOWER HEADS

White ray florets, yellow disk, odor, MATRICARIA.
Tubular and ligulate, yellow, odor, ARNICA.
Campanulate racemes, Convallariae Flores.

FLOWER BUDS

Dark red, 4 petaled globular head, calyx 4 toothed, odor, taste, CARYOPHYLLUS.

LEAF BUDS

Conical, imbricated scales, glossy brown, resinous, fragments, Populi Gemmae.

INFLORESCENCE

Purple clusters, tea like odor, Trifolium.

PARTS OF FLOWERS

Strobiles.
Yellow green, strong odor of hops, HUMULUS.
Trichomes.
Granular brown yellow powder, hop like odor, Lupulinum.
Pistillate panicles.

Green compact resinous masses, short branched flowers, CANNABIS.
Red brown masses or rolls, bitter taste, Brayera.
Florets.
Orange yellow, 1 to 3 toothed, 4 to 5 veined, Calendula.
Petals.
Cones or separate, purple red, odor, Rose Gallica.
Stigmas.
Single or in threes, rich red, cornucopia shaped, odor, Crocus.
Styles and stigmas.
Purple red to green yellow filaments, taste, Zea.
Corollas and stamens.
Light yellow, pubescent, three stamens, Verbasci Flores.
Pollen.
Yellow mobile inflammable powder, triangular grains, LYCOPODIUM.

FUNGI

Purple blue, triangular, curved, interior pink, ERGOTA.
Yellowish cakes or masses, starchy, yeast odor, Cerevisiae Fermentum Compressum.
Irregular gray to yellow, mealy, light pieces, fibrous, bitter, Agaricus.

ALGAE

Yellow to red, filiform flattened branched thallus, CHONDRUS.
Black brown dichotomous flattened cylindrical thallus, Fucus.

EXCRESCENCE

Green brown rounded tuberculated, very hard, GALLA.

THE LEAF

The leaf is the food manufactory and stomach of the plant. As an animal digests complex foods by means of various ferments, the leaf forms complex foods from simple elements and digests them.

Starch is formed by sunlight acting on the chlorophyll corpuscles, along with carbon dioxide and water; this process is called "photosynthesis."

The leaf is usually covered with a transparent skin, the epidermis, somewhat cutinized externally in some cases, having no intercellular spaces, hence impervious to atmosphere changes and water. Flat sections show many wavy outlined epidermal cells, more or less characteristic, and of various shapes and sizes. Some of the epidermal cells are modified into trichomes and stomata. The stomata consist of two crescent shaped cells—the "guard cells," having concave surfaces and containing coloring matter, the only place in the epidermis where coloring matter is found. The stomata are respiratory organs, for gases as carbon dioxide and oxygen. They also regulate the evaporation of liquids and excretions, and vary in shape from oval to oblong. They are capable of opening and closing. Usually a definite number of epidermal cells surround each stoma in the various plants. These cells are called "neighboring" cells. Water pores are found on the lower surfaces and are for transpiration-exhalation of water, which also takes place through the stomata.

The trichomes are modified epidermal cells, their function in the leaves being protection, mechanically or by means of their secretions, acids, odoriferous oils or resins. The shapes of these appendages vary as seen on the page describing trichomes. Trichomes are of the utmost importance in diagnosing powdered leaves.

The fibro-vascular tissue is the support or framework—the mid rib and veins, and for conduction of liquids, and consists of wood, bast, stone, tracheids, tracheae, laticiferous and sieve. The kind of vessels often aid in diagnosing a leaf.

The fundamental tissue consists of two main types of tissues—in the leaf the fundamental tissue is called the mesophyll, palisade arranged at right angles to the surface and the spongy parenchyma—a very irregular variety of parenchyma, with large intercellular spaces. Both varieties are rich in chlorophyll corpuscles—which contain the chloroplastids. These bodies become active in the sunlight and are the starch forming bodies. Starch is at first formed, then with the aid of digestive ferments, the starch is digested into a sugary solution, which is then carried to the plant store houses or to the plant parts needing nutrition. Crystals and oil are also found in the mesophyll.

Leaves may be divided into:

1. Bi-facial; as the name implies, the leaves have two different surfaces. The bi-facial leaves are the most common leaves. The stomata are usually on the upper surface—

ventral," and sometimes only on the lower—"dorsal" surface, and sometimes on both surfaces. The epidermal cells of the dorsal surface are less cutinized externally than those of the ventral surface. The upper surface may have an extra protecting layer of cells, the "hypoderm"—a layer of thick walled modified epidermal cells, beneath the epidermis. Palisade cells usually are beneath the upper surface. Collateral fibro-vascular bundles are most frequent, the xylem facing the ventral surface.

2 Centric leaves as in the pines, and Calamus, show but little differentiation between the two surfaces. Palisade cells are rare or modified. In the leaves of the gymnosperms, tracheids with bordered pores replace the vessels and wood cells. An endoderm may surround the bundles. The petiole usually contains a large bundle.

Diagnostic Points of Some Leaves.

Tea is diagnosed by having peculiar irregular stone cells—idioblasts, cane shaped hairs, and rounded stomata. The epidermis of all Teas show on a transverse view, a beaked or hooked appearance, due to the prolongation of the cutinized inner surface of the guard cells. Cameilla, one of the adulterants, on a transverse section show little development of the guard cells into the hooked appearance.

Cannabis is characterized by the cystolith—retort shaped trichomes containing calcium carbonate in their bases.

Belladonna by having at least three different shaped calcium oxalate crystals and the characteristic trichomes.

Digitalis is the only narcotic leaf free from crystals and starch. This is uncommon in leaves. Digitalis has a collapsed trichome—next to the end cell collapsed.

Mentha Piperita—various shaped trichomes, a collapsed trichome—the end cell is collapsed or the end cell and the third cell from the end collapsed. Characteristic glandular trichomes.

Hyoscyamus has at least four different shaped crystals, and the "copper head snake" shaped trichome. The flower anthers and pollen give a violet color with chloral test solution.

Tobacco with the "crab claw" shaped hair and sphenoidal micro-crystal cells (also found in Belladonna, Hyoscyamus, Stramonium and Cannabis).

Stramonium-parenchyma and palisade with chloroplasts and crystals of various shapes, the diagnostic glandular trichome and elliptical stomata. No bast. In Xanthium Stramonium, no Stramonium alkaloids are found.

Eucalyptus with about 90% of its cells palisade. Peculiar schizogenous oil cells and many large intercellular spaces due to the separation of the palisade. Four different shaped crystals. No trichomes.

Pilocarpus has palisade occupying about 1-5 the thickness of the leaf. Diagnostic trichomes, oil cells, stone, crystals and bast.

Buchu has crystals of hesperidin and mucilage in the epidermal cells.

Salvia has the one to five celled trichomes filled with air, on both surfaces.

Senna—Indian—elliptical or elongated stomata, few or no hairs or crystals. Alexandria—rounded stomata, many characteristic hairs and crystals. Crystal fibers.

Matico has no glandular trichomes, but many simple characteristic trichomes, oil cells with yellowish corky walls. Crystals.

Eupatorium. Characteristic trichomes. Eupatorium glutinosum has long simple twisted trichomes, and many short glandular trichomes.

Eriodictyon is diagnosed by the "cork screw" shaped trichomes.

Mentha Viridis has diagnostic compound and simple trichomes.

Scutellaria is characterized by having the peculiar shaped stomata, and the simple and compound trichomes.

Coca has a papillated or saw toothed like epidermis when seen on a transverse view. Few or no trichomes. Epidermal cells are five to six sided.

Hamamelis—colorless stone, trichomes in groups of about fifteen.

Lobelia has cells with inulin, the stomata surrounded by three to four neighboring cells.Characteristic trichomes.

Marrubium is diagnosed by the somewhat "T" shaped trichomes and glandular trichomes.

Malva has the long, large thick walled curved trichomes and the glandular short stalk trichomes. Crystals and mucilage.

Chimaphila epidermal cells covered with resin. Porous lower epidermal cells.

Inula contains inulin, and has the characteristic trichomes.

Castanea has simple, thick walled, slightly curved trichomes in groups of about five. Crystal bast.

Althaea has diagnostic trichomes in groups of two to more than five.

Grindelia with the sessile glandular trichomes in depressions of the epidermis. Many resin cells near the mid rib.

Rosa Gallica is characterized by the two different diagostic epidermises, coloring matter in the cells, the trichomes nd papillated epidermis.

Anthemis has characteristic trichomes.

Some of the leaves having:

Thick outer epidermal walls—are Uva Ursi, Pilocarpus, Eucalyptus and Chimaphila.

Thin outer epidermal walls—are Belladonna Hyoscyamus, Mentha Piperita, Digitalis and Marrubium.

Single row of palisade on the upper side—are Hamamelis, Stramonium, Coca and Mentha Piperita.

Single rows of palisade on upper and lower sides—is Senna.

Composed entirely of palisade except for resin and fibrovascular bundles—is Eucalyptus.

Branching spongy parenchyma—is Stramonium.

Many rosette crystals of calcium oxalate—is Stramonium.

Many prism crystals—are Hyoscyamus, Coca, Senna and Eucalyptus.

Sphenoidal micro-crystals—are Belladonna, Cannabis, Tobacco and Stramonium.

Stone cells—are Hamamelis and Tea.

Secretion cells—Buchu and Eucalyptus.

Crystal fibers—are Senna and Coca.

Glandular trichomes—are Senna and Hamamelis.

Glandular and non-glandular trichomes—are Tobacco, Belladonna, Hyoscyamus, Mentha Piperita, Mentha Viridis, Stramonium, Cannabis, Matico and Eriodictyon.

Non-glandular trichomes—are Tea, Pilocarpus, Senna, Hamamelis, Chimaphila, Castanea and Althaea.

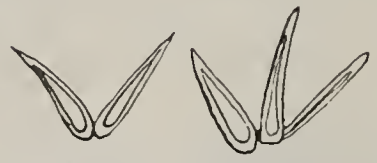
No trichomes—are Eucalyptus, Coca and Buchu.

Kinnikinic the leaves or bark of certain plants as the willow or sumac prepared for smoking.

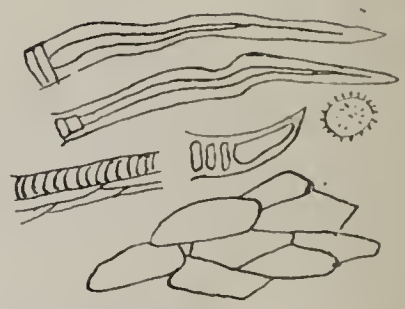
EXERCISE

Make flat and tv. sections of Eucalyptus, Buchu, Senna, Cannabis, Hyoscyamus, Stramonium, Digitalis and Belladonna. Study for contents, then for structure. Unless mucilage is present, sections are readily made by softening the leaf in water, placing the softened leaf between two glass slides and cutting the transverse section; by boiling the leaf in water until soft and wrapping the softened leaf over the

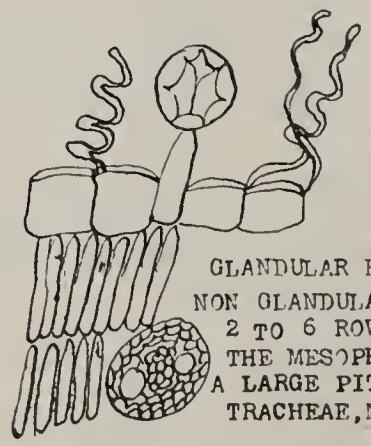
index finger, the flat section may easily be picked off with the teasing needle. Be careful that no green shows on the flat section or it will be too thick to examine for structure. A good workman is known by his tools; you cannot do good work with dull, rusty or dirty instruments. After you have made the tests for cell contents and have drawn the sections and named ALL the cells, state if trichomes are present or not, also the kinds and illustrate; also give the tests and results for any characteristic cells or cell contents; draw diagnostic cells. Ask for the powder of the leaf you are studying and with aid of your U. S. P., answer the following questions: Name and draw ALL the cells; test and give reactions for the cell contents; note any diagnostic cells or cell contents; name the chief adulterants and state how they are identified.



QUERCUS INFECTORIA

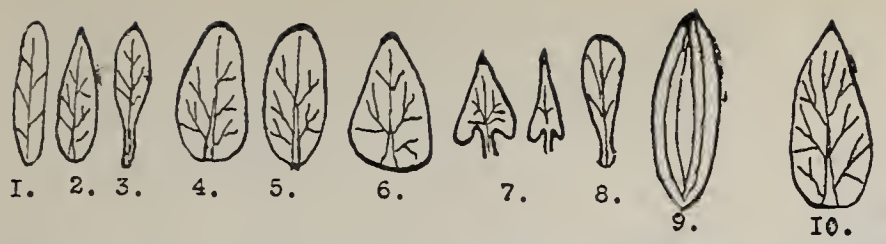


ANTHEMIS.



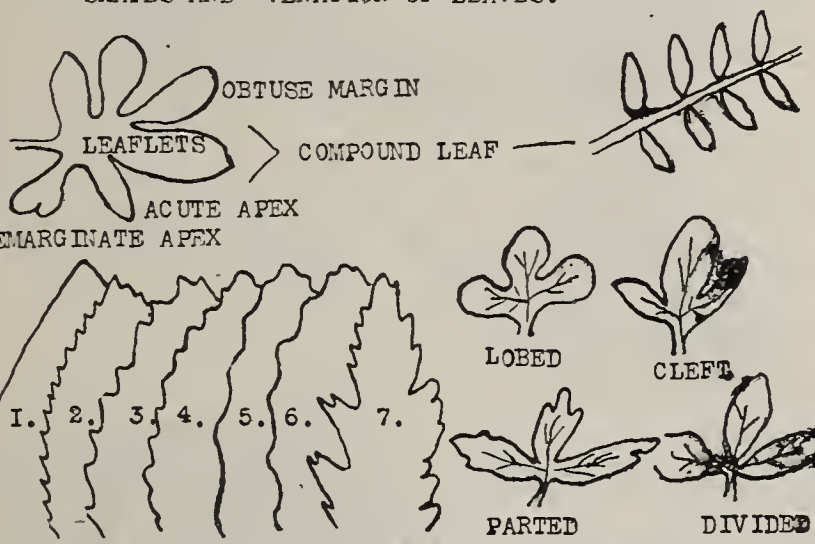
GLANDULAR HAIRS 6 TO 8 CELLED HEAD.
NON GLANDULAR HAIRS CORK SCREW SHAPED.
2 TO 6 ROWS PALISADE. FEW CELLS IN
THE MESOPHYLL. THE MID RIB CONTAINS
A LARGE PITH, BAST, WOOD, SIEVE AND
TRACHEAE, MEDULLARY CELLS ONE CELL WIDE.

ERIODICTYON.



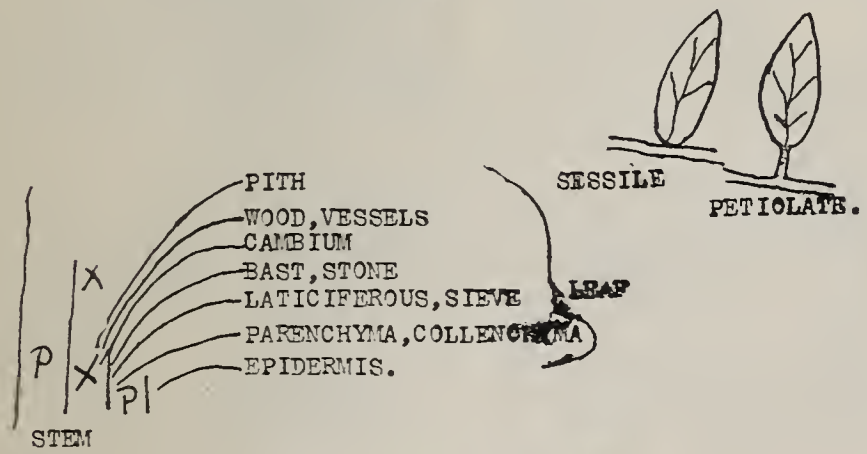
I. LINEAR, 2. LANCEOLATE, 3. OBLANCEOLATE, 4. OBLONG. 5. ELLIPTICAL
6. OVATE, 7. SAGITTATE, 8. SPATULATE, 9. PARALLEL-VEINED,
IO. RETICULATE-VEINED.

SHAPES AND VENATION OF LEAVES.

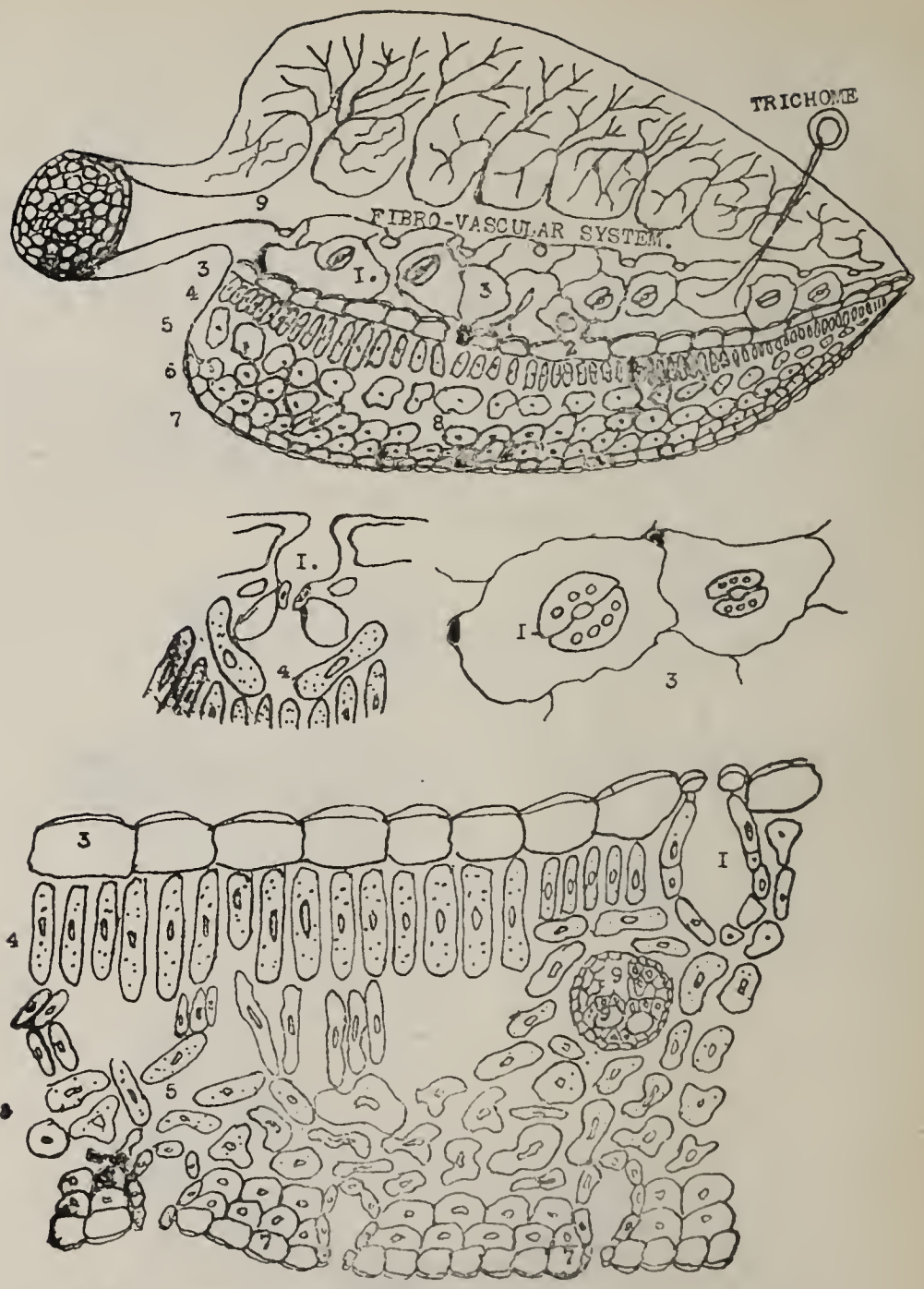


I. WHOLE, 2. SERRATE, 3. DENTATE
4. CRENATE, 5. REPAND, 6. SINUATE, 7. INCISED.

MARGIN OF LEAVES.

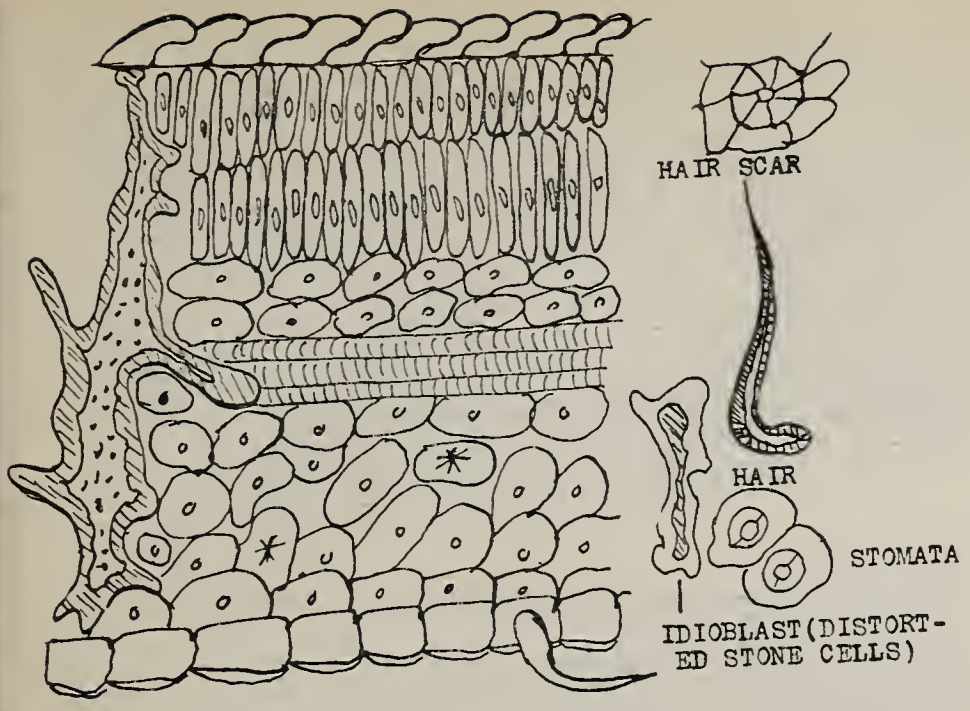


RELATION OF A STEM AND A LEAF.

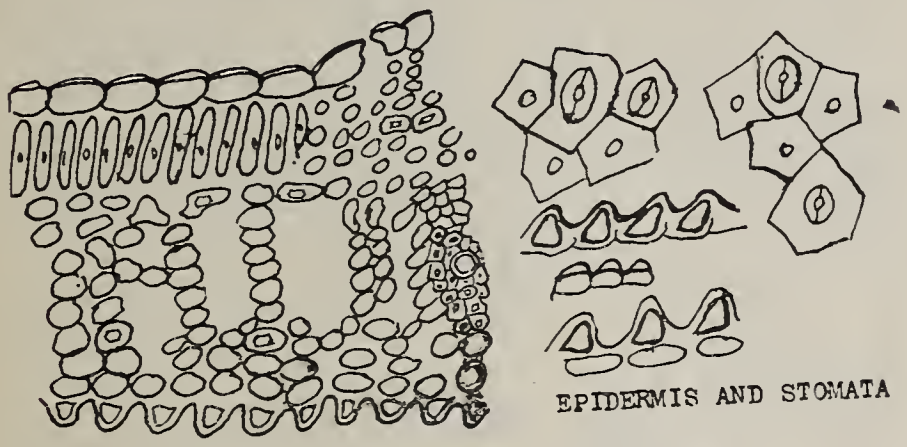


I. STOMATA, 2. WATER PORES, 3. UPPER EPIDERMIS, 4. PALISADE, 5. SPONGY PARENCHYMA, 6. COLLENCHYMA, 7. LOWER EPIDERMIS, 8. MESOPHYLL, 9. MID RIB OR THE FIBRO-VASCULAR SYSTEM.

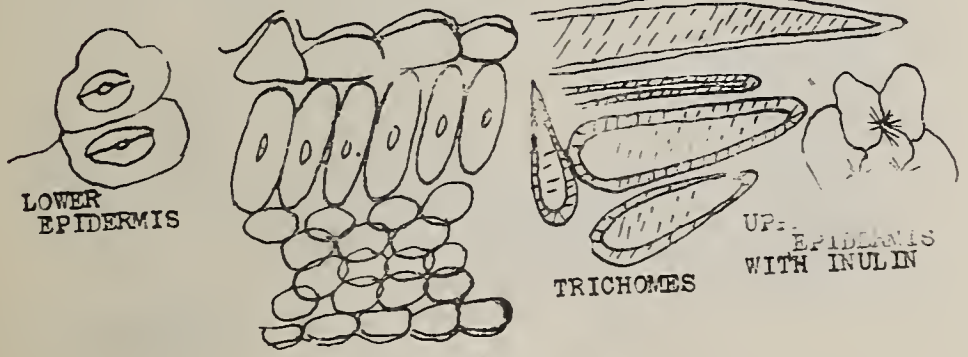
THE STRUCTURE OF A TYPICAL LEAF.



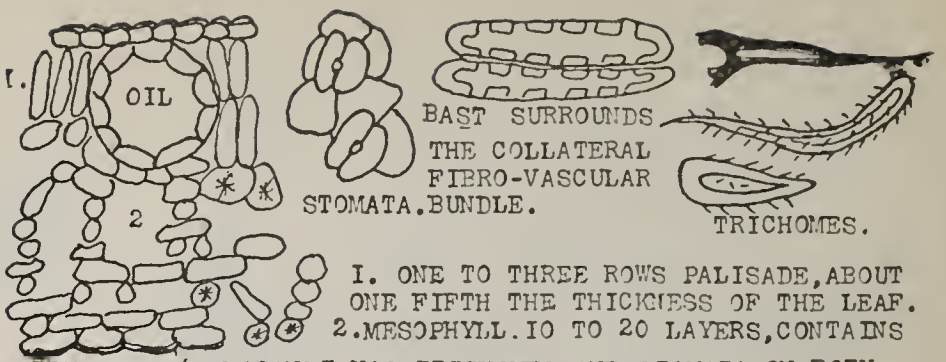
T E A .



C O C A .

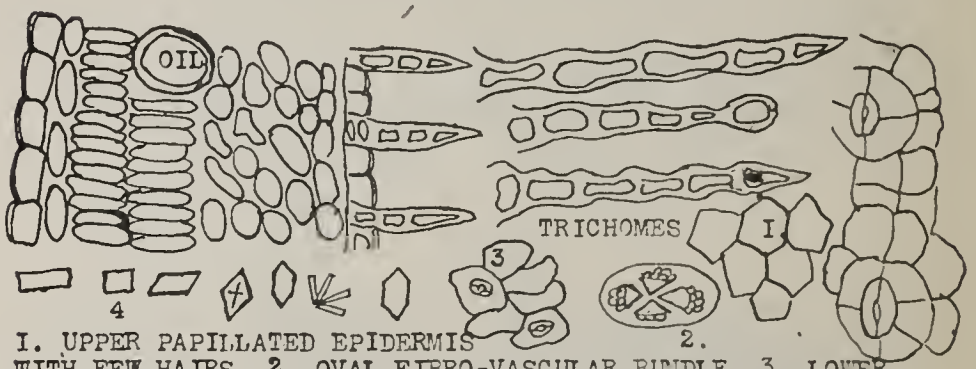


L O B E L I A .



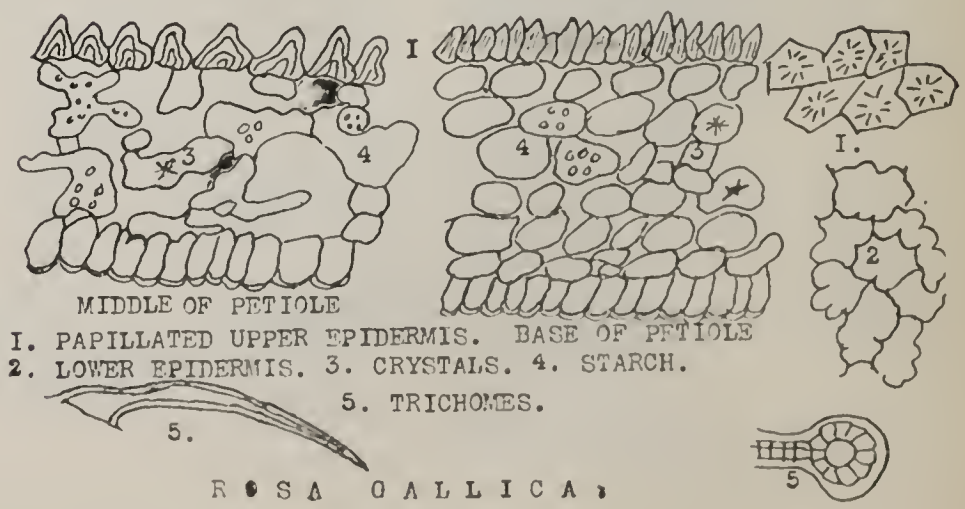
I. ONE TO THREE ROWS PALISADE, ABOUT ONE FIFTH THE THICKNESS OF THE LEAF. 2. MESOPHYLL. IO TO 20 LAYERS, CONTAINS CRYSTALS. (JABORANDI HAS TRICHOMES AND STOMATA ON BOTH SURFACES.) THE UPPER EPIDERMIS IS CUTINIZED.

P I L O C A R P U S . (MICROPHYLLUS)



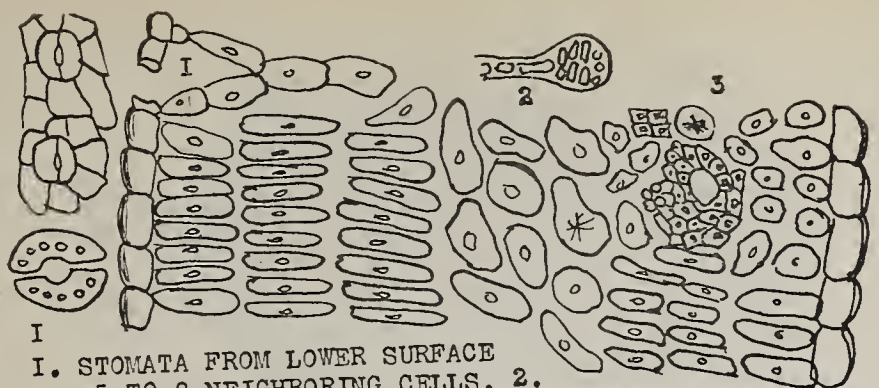
I. UPPER PAPILLATED EPIDERMIS WITH FEW HAIRS. 2. OVAL FIBRO-VASCULAR BUNDLE. 3. LOWER EPIDERMIS WITH 6 TO 8 NEIGHBORING CELLS, AND MANY POINTED BENT BRISTLY THICK WALLED, STRIATED, TRICHOMES. PALISADE 2 TO 3 ROWS.

M A T I C O .



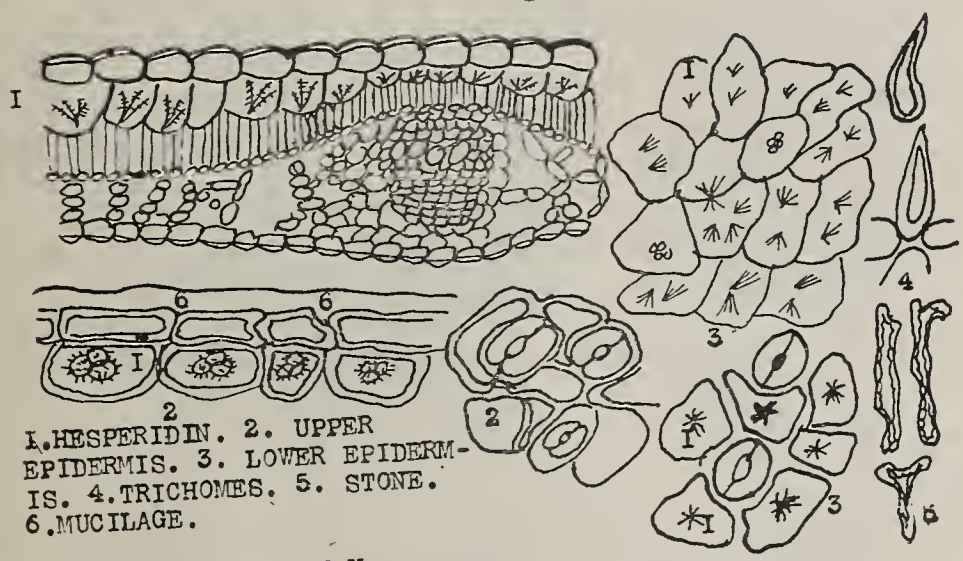
I. PAPILLATED UPPER EPIDERMIS. 2. LOWER EPIDERMIS. 3. CRYSTALS. 4. STARCH. 5. TRICHOMES.

R O S A G A L L I C A .



I. STOMATA FROM LOWER SURFACE WITH 5 TO 8 NEIGHBORING CELLS. 2. TRICHOME FROM THE STEM. 3. CRYSTAL FIBERS. THE MESOPHYLL CONTAINS CHLOROPLASTS, A CARBOHYDRATE, AND A YELLOW BROWN CONTENT. THE POWDERED DRUG SUBLIMED ON A DISH SLIDE YIELDS LONG FEATHERY CRYSTALS.

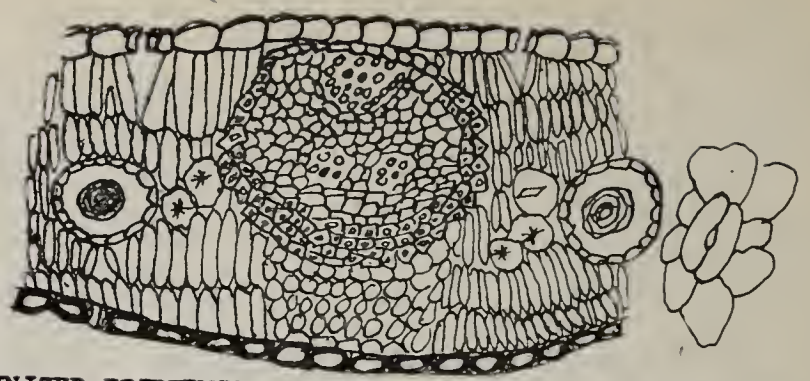
U V A U R S A .



1.HESPERIDIN. 2. UPPER EPIDERMIS. 3. LOWER EPIDERMIS. 4.TRICHOMES. 5. STONE. 6.MUCILAGE.

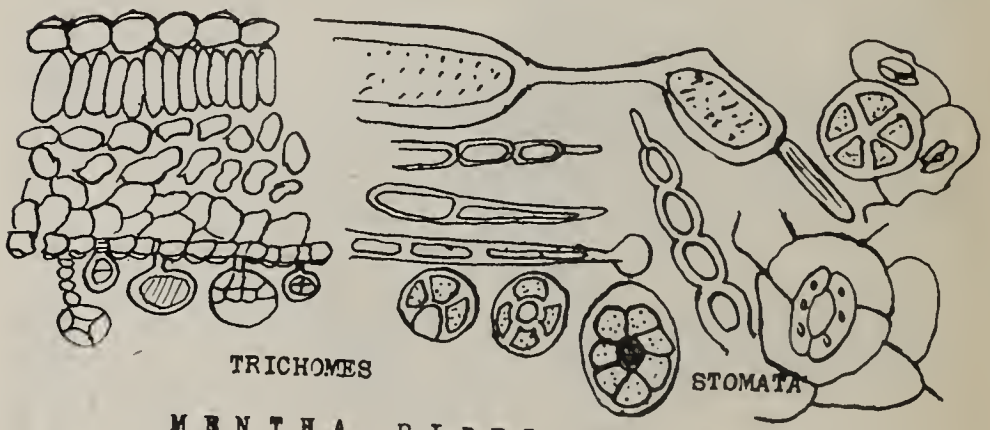
B U C H U .

A L T H A E A F O L I A .

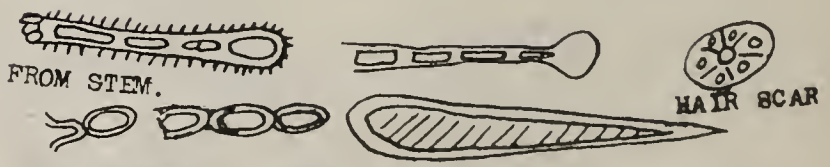


CUTINIZED EPIDERMIS, MANY RESIN AND CRYSTAL CELLS, SOME BAST LIGNIFIED, 90 % OF THE CELLS ARE PALISADE, MANY STOMATA ARE SUNKEN, OTHERS FILLED WITH RESIN.

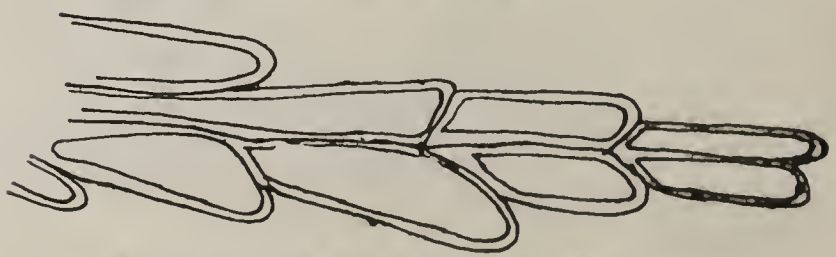
E U C A L Y P T U S .



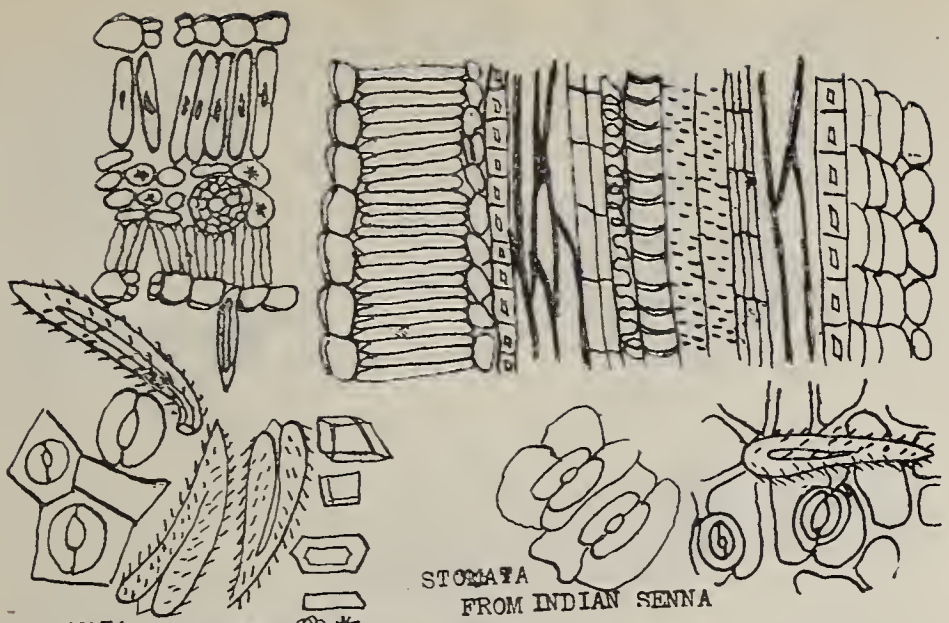
M E N T H A P I P E R I T A .



MENTHA VIRIDIS (SPICATA)

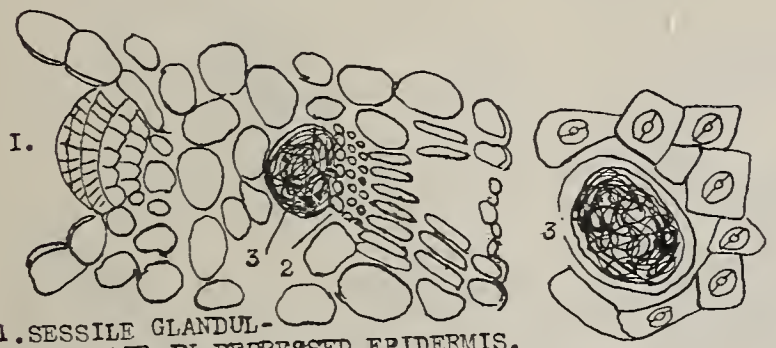


E U P A T O R I U M TRICHOMES.



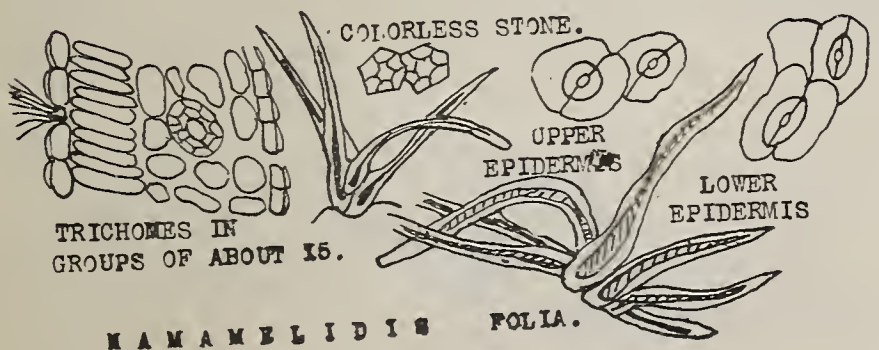
STOMATA, TRICHOMES AND CRYSTALS IN ALEXANDER SENNA.

S E N N A .



1. SESSILE GLANDULAR HAIR IN DEPRESSED EPIDERMIS.
2. THE MID RIB 3. RESIN CELLS.

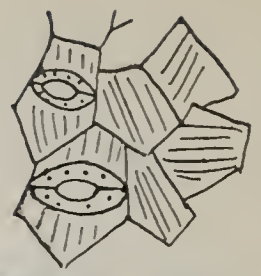
G R I N D E L I A .



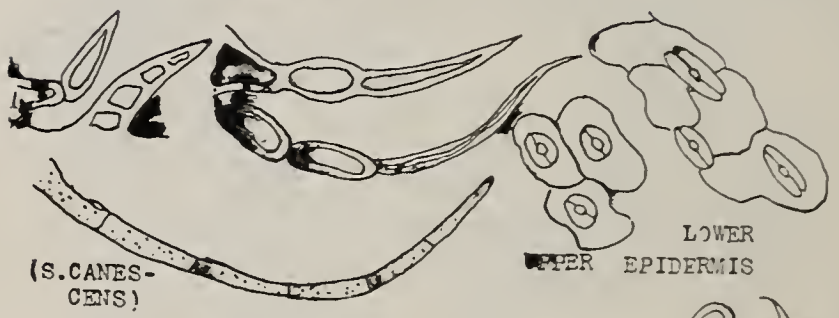
H A M A M E L I D I S FOLIA.

CELLED GLANDS.

MAJORAM.

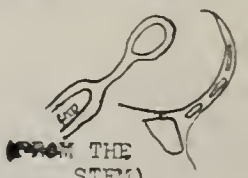


ARI (MYRTIFOLIA)
SONCUS ADULTERANT
FOR MAJORAM.



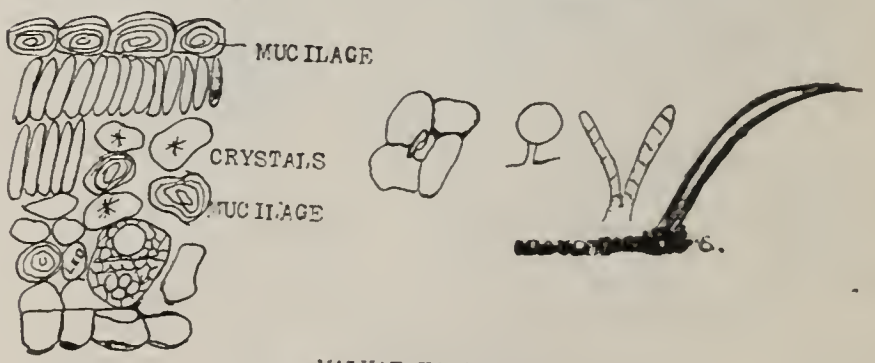
(S.CANES-
CENS)

LOWER
EPIDERMIS



THE
STEM)

SCUTELLARIA.



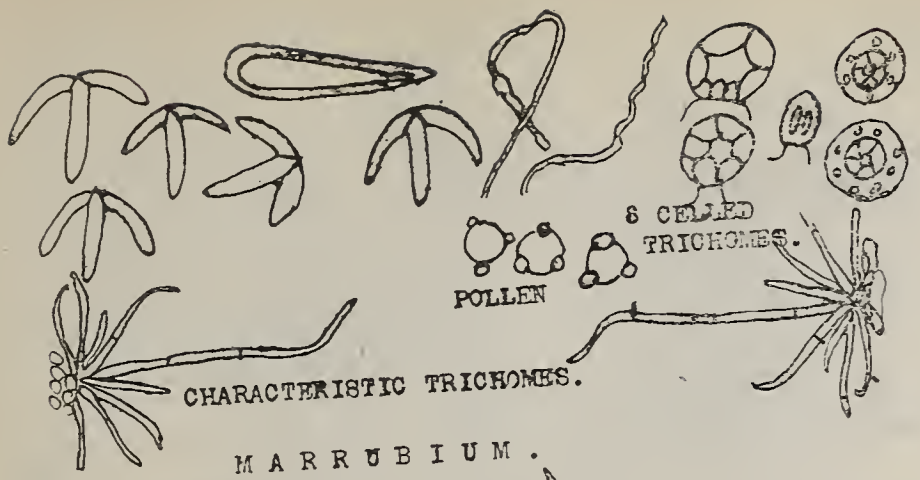
MUCILAGE

CRYSTALS

MUCILAGE

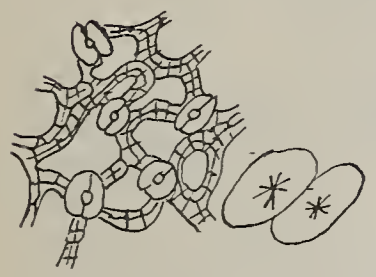
MALVAE FOLIA.

TRICHOMES FROM
SOLANUM CAROLINIANUM

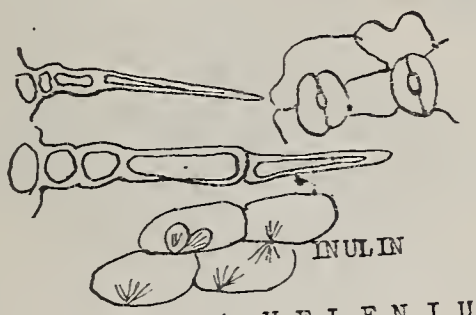


M A R R U B I U M .

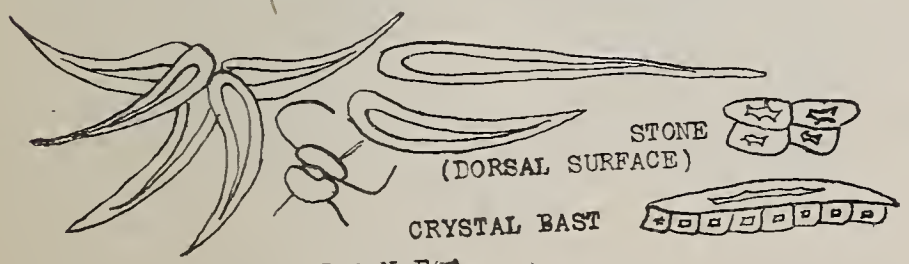
ADULTERANTS OF MARRUBIUM



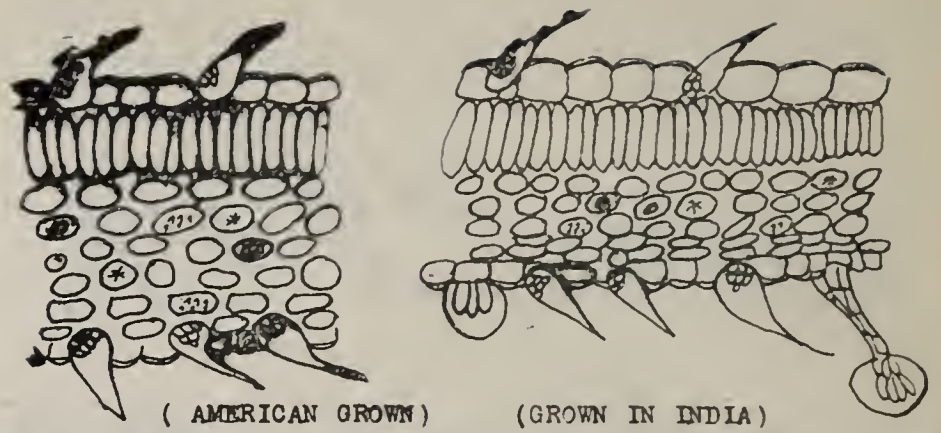
C H I M A P H I L A .



I N U L A H E L E N I U M .



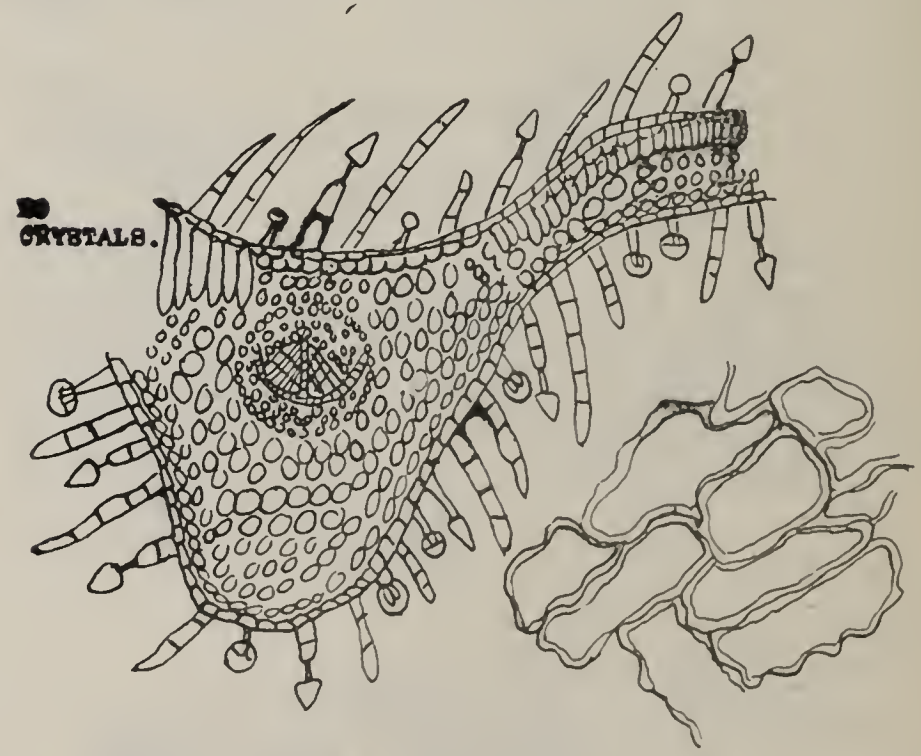
C A S T A N E A .



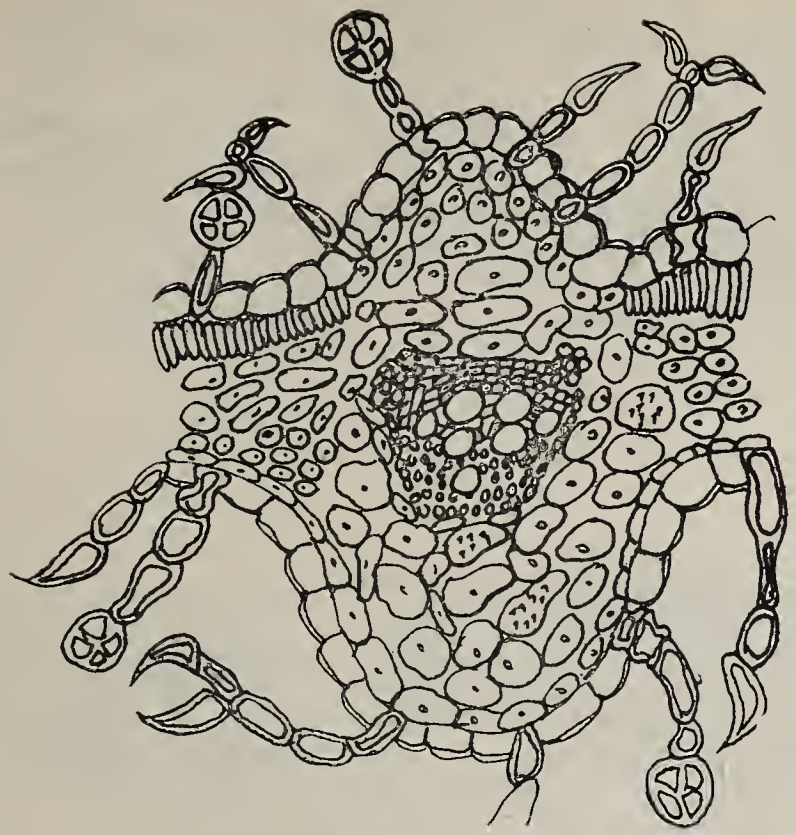
(AMERICAN GROWN) (GROWN IN INDIA)

BOTH CANNABIS CONTAIN IN THE MESOPHYLL, MUCH FIXED OIL, RESIN CRYSTALS, CRYPTO-CRYSTALLINE OR SPHENOIDAL MICRO CRYSTAL CELLS AND THE RETORT SHAPED HAIRS WITH CALCIUM CARBONATE IN THEIR BASES.

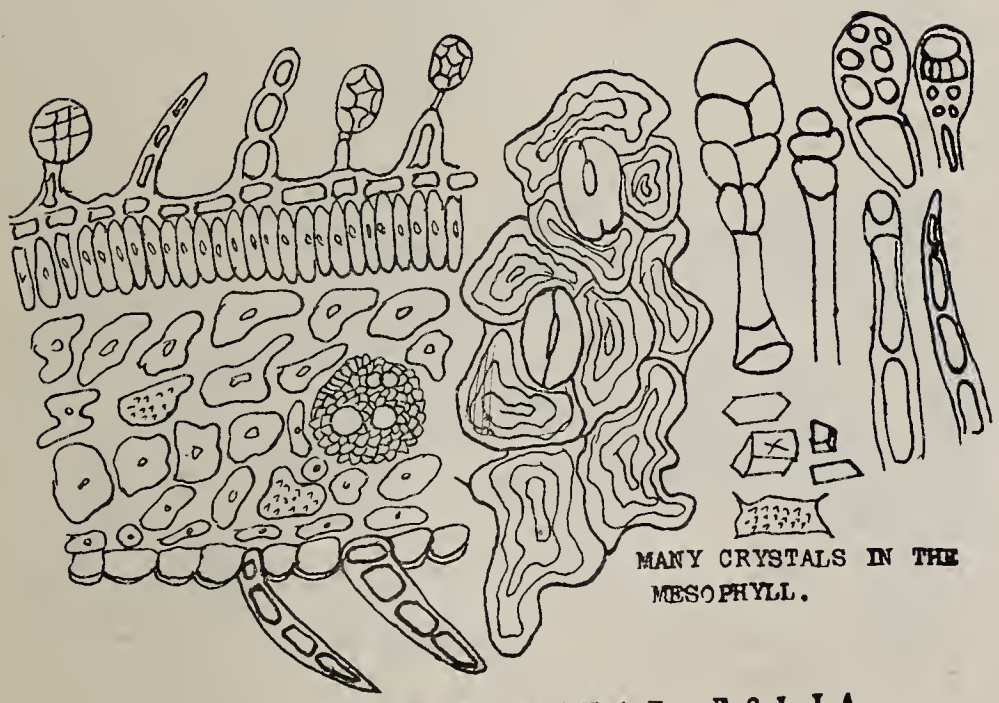
C A N N A B I S .



D I G I T A L I S .

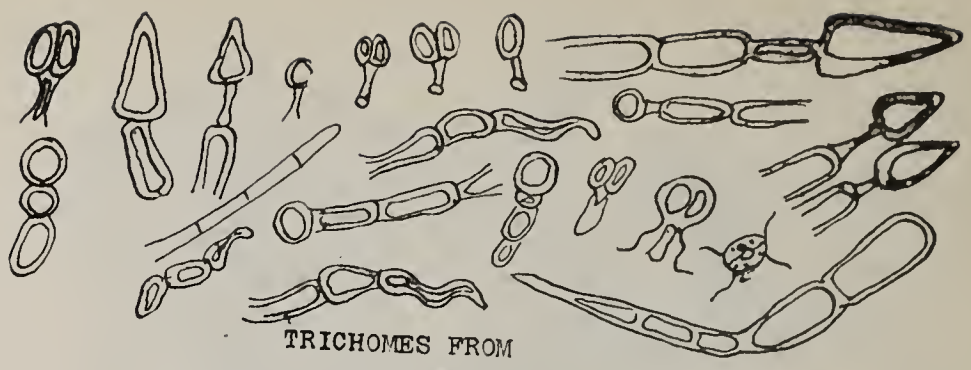


TOBACCO.



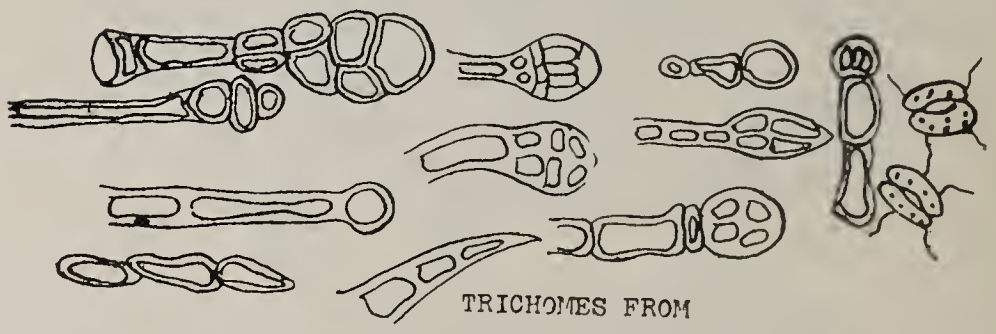
MANY CRYSTALS IN THE MESOPHYLL.

BELLADONNAE FOLIA.



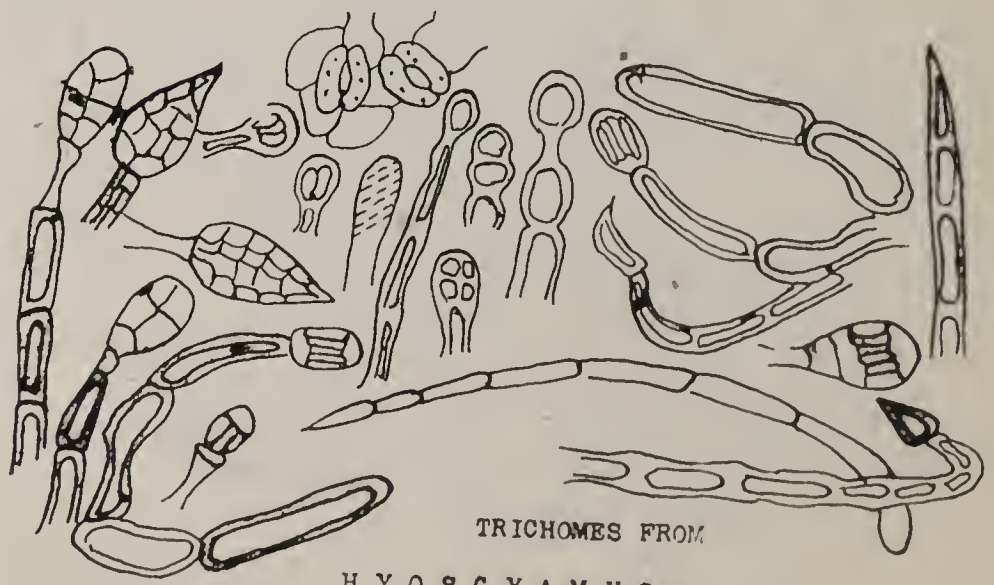
TRICHOMES FROM

DIGITALIS.



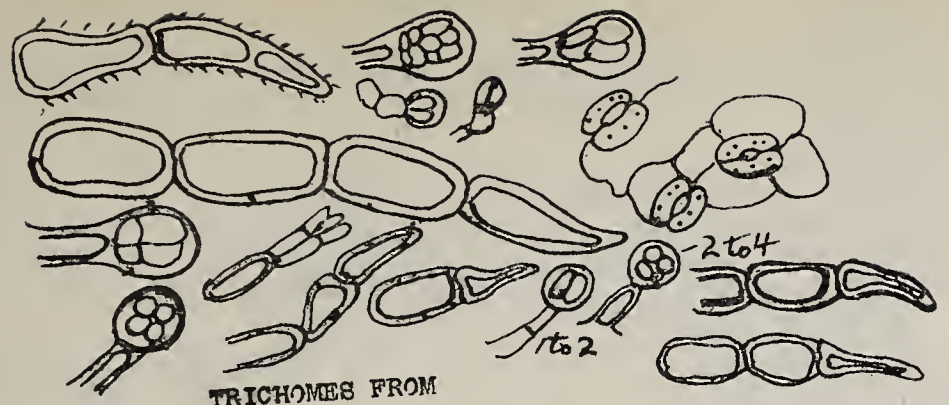
TRICHOMES FROM

BELLADONNAE FOLIA.



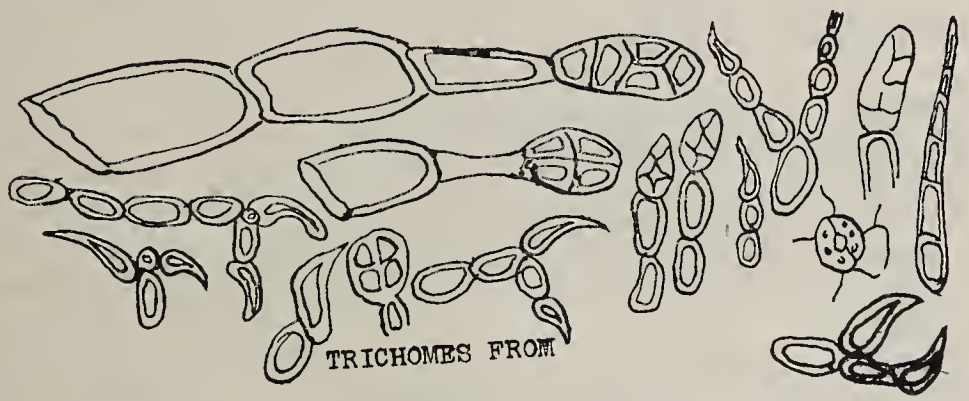
TRICHOMES FROM

HYOSCYAMUS.



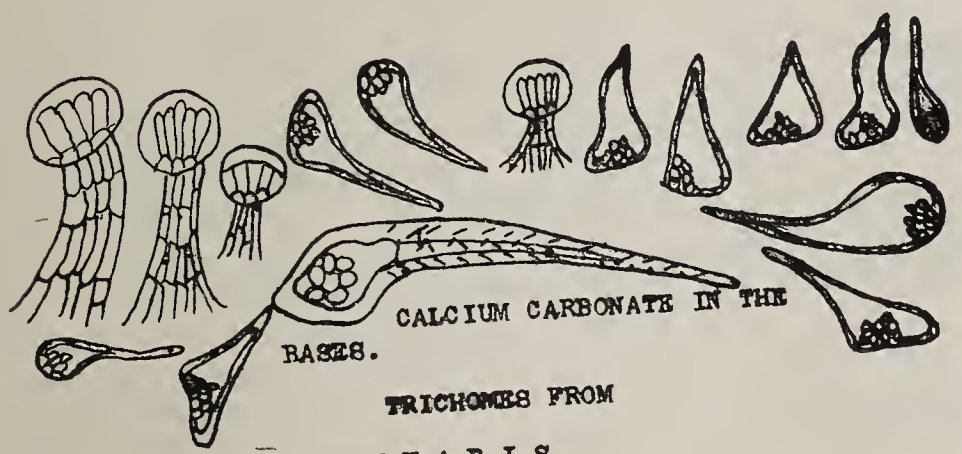
TRICHOMES FROM

STRAMONIUM.



TRICHOMES FROM

TOBACCO.



CALCIUM CARBONATE IN THE BASES.

TRICHOMES FROM

CANNABIS.

FRUITS

A fruit is a fertilized and ripened ovary or sometimes with attending parts, composed of Epicarp, Mesocarp and Endocarp.

Fresh fruits.

Drupes. A carpelled fruit, as cherry or prune, consisting of Exocarp, skin; Mesocarp, the succulent portion, and Endocarp, the hard seed. Indehiscent.

Reddish to blackish, 30 to 40 drupes.

Blackberries, Rubi Fructus.

Raspberries, Rubi Idaei Fructus.

Dry or cured fruits.

Drupes.

Globular, reticulate marked, peduncle, slender, dry, from 3 to 7 mm., taste, CUBEBA.

Globular, wrinkled, 3 to 4 mm., dry, no peduncle, taste, PIPER.

Oval, thin wrinkled epicarp, sweet sarcocarp, taste. Prunum.

Coriaceous wrinkled, rounded, acrid taste, SABAL.

Rounded, reniform, brown black, wrinkled, acrid taste, Cocculus Indicus.

Round, wrinkled, blackish, internally green, sections are horse shoe shaped. Rhamni Catharticae Fructus.

Globular, smooth, brittle, 4 toothed calyx, odor and taste, Pimenta.

Rounded, reddish, hairy, astringent, Rhus Glabra.

Capsules or pods. Dry dehiscent fruits, containing many seeds.

Dark brown, flexible pod, 15 to 25 cm. long, odor, Vanilla.

Rounded, elongated, pale brown, brittle, many small seeds, Papaveris Fructus.

Cylindrical pod, chocolate brown, 25 to 100 ovate hard seeds, Cassia Fistula.

Fleshy yellow brown pods, 1 or 2 black subglobular seeds, Xanthoxyli Fructus.

Cremocarps. A double fruit of the Umbellifera family, each half called a mericarp resembling an achene which is a small 1 seeded hard dry indehiscent fruit.

Oval, hairy, 5 ribs, 16 to 30 oil tubes, odor, taste, ANISUM.

Globular, smooth, hollow, 9 ribs, 2 oil tubes, odor, taste, CORIANDRUM.

Curved, elongated, 5 ribs, 5 to 6 oil tubes, odor, taste, CARUM.

Oval, greenish brown, 5 ribs, 2 grooves, no oil tubes, Conium.

Oval, brown, smooth, 5 ridges, 12 oil tubes, spicy odor, Anethi Fructus, N. O.

Oblong, pale brown, cylindrical, 5 ribs, 4 to 8 oil tubes, odor, FOENICULUM.

Oblong, yellow brown, 3 ridges, 4 grooves, 12 oil tubes, Cumini Fructus.

Round ovate, gray green, 5 ribs, 6 oil tubes. PETROSELINUM.

Round, ovate, compressed, 5 ribs, 12 to 16 oil tubes. Apii Fructus.

Ovate elliptical, flattened, yellowish, 5 ribs, many oil tubes, Angelicae Fructus.

Fleshy fruits.

Berry. A thin membranous pericarp, succulent many seeded fruit as grapes or tomato.

Oblong, conical, reddish pericarp, pungent taste, CAPSICUM.

Galbulus. A succulent berry-like cone.

Rounded, wrinkled, blackish, about 5 mm., Juniperus.

Oval, orange brown, wrinkled, bitter and acrid, Solanum.

Syconium. A product of flower clusters, or collective fruits.

Pear shaped when fresh, compressed when dry, taste, Ficus.

Parts of fruits. (Fruits).

Pulp.

Black brown masses, sweet acid taste, Tamarindus.

Yellow white, soft spongy masses, mixed with seeds, very bitter, COLOCYNTHIS.

Pericarp.

Dry, brown green quarter or ribbon sections, odor, taste, AURANTII AMARI CORTEX.

Dry lemon yellow spiral bands, odor, taste, LIMONIS CORTEX.

Fresh quarters, orange yellow, odor and taste, AURANTII DULCIS CORTEX.

Fecula.

White powder, odorless and tasteless, AMYLUM.

SEEDS

The fertilized and ripened ovule, consisting of the Testa, Tegmen and Embryo, the embryo composed of the cotyledons, etc.

Large seeds.

Ellipsoidal, brownish, wrinkled, interior marbled, odor, taste, MYRISTICA

Orbicular, flat, gray disks, depressed center, hard, hairy, NUX VOMICA.

Obovate oblong, taste sweetish, AMYGDALA DULCIS.

Ovate, angular, brownish black, hard, Ignatia.

Reniform, red brown, groove on convex side, PHYSOSTIGMA.

Oval, flat, yellow white, marginally grooved, PEPO

Oblong lanceolate, yellow green to brown, silky hairs, STROPHANTHUS.

Plano convex, flat side grooved, brownish, odor, taste, Caffea Tosta.

Triangular, reticulate wrinkled, brownish, STAPHISAGRIA.

Ovate, flat, smooth, mucilaginous, brownish, LINUM.

Small seeds.

Angular, red brown, pitted, odor,
Globular.
CARDAMOMI SEMEN.

Yellow brown, finely pitted, odor on moistening, SINAPIS ALBA.

Blackish, oily, strong odor on moistening, SINAPIS NIGRA.

Ovoid, dark brown, pitted, very hard, COLCHICI SEMEN.

Tetrahedral, warty, blackish, internally whitish, oily, Delphinium.

Parts of seeds.

Cotyledons.

Plano convex, rounded, brownish black, very hard, Kola.

Hairs.

Masses of thick edged twisted ribbon like hairs, GOSSYPIUM PURIFICATUM.

Paste.

Red brown, chiefly crushed seeds, odor, GUARANA.

Arilla.

Thin irregular or cleft bands, odor, Macis.

Prepared.

Reddish brown powder, chocolate taste, Cacao Praeparata.

Yellow amber grains, crisp fracture, interior whitish, MALTUM.

FRUITS AND SEEDS

A fruit is a fertilized and ripened ovary. A seed is fertilized and ripened ovule. They are classified as to their contents, as "albuminous" (those having an extra albuminous food supply, using the perisperm or endosperm or both as store houses). and "exalbuminous," which have no extra albuminous food, as in Pea, Pumpkin, et al.

In structure fruits and seeds resemble somewhat that of leaves, but less lignified.

The parts of fruits and seeds are:

Micropyle—the scar at the apex, the point of attachment

Hilum—where the seed has left the funiculus.

Raphe—the ridge.

Seed coats, Testa—the thin brown outer scurf, easily separated as in Almond. Tegmen—inner silvery membrane.

Nucellus—white and oily ovule or embryo.

Embryo—the plantlet within the seed, always free and visible, and composed of Cotyledons—the part which becomes the first leaf or leaves.

Perisperm—store house outside the nucellus.

Endosperm—store house outside of the embryo.

Classified as to embryo:

Embryo—alone as in Almond.

Embryo—surrounded by endosperm as in Linum, Nux Vomica and Strophanthus.

Embryo—surrounded by endosperm and perisperm as in Cardamomum.

Microscopical appearance:

The cotyledons are colorless, showing the form of a leaf, with a modified epidermis, and composed almost entirely of very small palisade cells, through which is a rudimentary fibro-vascular bundle. Endosperm contains parenchyma rich in food materials. There are no vessels in the perisperm or endosperm, and both are used for storage of foods, as albumens, carbohydrates, fats and starches—these are the foods most commonly found.

The contents are of great importance, many times more so than the structure. Oils are the principal foods of seeds, occurring as rounded masses or droplets. Albumen occurs as aleurone, proteid bodies and crumbly masses.

Classification of the tissues:

Thick walled, little or no starch, as Nux Vomica, Colchicum, Coffee, Sabadilla.

Tissues with starch, as Quercus, Guarana, Physostigma and the Legumes.

Thick walled tissues with fats:

a. With endosperm, as Linum and Strophanthus.

b. No endosperm, as Sinapis.

	Water	Glycerin	KOH
Starch	Insoluble	Insoluble	Clump
Fat	Insoluble	Insoluble	Saponify
Albumen	Partly	Partly	Partly
Inorganic Crystals	Insoluble	Insoluble	Insoluble
	Alcohol	Ether	Iodine
Starch	Insoluble	Insoluble	Blue to black
Fat	Insoluble	Soluble	Brownish
Alubmen	Insoluble	Insoluble	Brownish
Inorganic Crystals		Insoluble	

Fats, and fixed oils turn red with Tr. Alkanet, and black with Osmic acid. Starch in small amounts is very common to seeds.

EXERCISE

Make transverse sections of Pepo, Nux Vomica, Physostigma, Strophanthus, the Mustards, Cubeba and Foeniculum. Study for contents, then for structure. Proteid bodies stain brown with iodine—the accompanying spheres are calcium and magnesium phosphates. Examine the cotyledon and determine whether the parenchyma is thin walled, and whether there are delicate spiral vessels—young seed; or if the parenchyma is thick walled, which indicates an old seed. Most U. S. P. seeds and fruits contain stone cells, aleurone and starch. These are of great importance in the identification of the drugs.

Diagnostic Points of Some Fruits and Seeds

Colchici Semen has two to six compound spherical, oval, or polygonal starch grains, with the hilum triangular or forked.

Linum contains, red brown pigment cells, stone cells, fixed oil, aleurone, and a cutinized epidermis with mucilage.

Guarana is characterized by the single, double or triple starch and crystals.

Coffee has the twin stone cells.

Sinapis Alba has goblet shaped cells and mucilage.

Sinapis Nigra has pigment cells, stone cells and mucilage.

Nux Vomica has a lignified needle shaped hair coming from every epidermal cell.

Physostigma has the slender "test tube" shaped palisade, and many reniform shaped starch.

Strophanthus is diagnosed by the trichome. If Strophanthus is exhausted of the glucoside, sections or the powder will show, when sulphuric acid (80%) is added, the trichomes clumped and the embryo brownish. If not exhausted, the trichomes are not clumped and are of a reddish color, and the embryo greenish. There is little or no starch or calcium oxalate in Strophanthus. Some of the unofficial allied species, contain much calcium oxalate in the embryo, and with sulphuric acid the embryo gives a red color.

Pepo has the lignified palisade shaped epidermis, stone and large spongy parenchyma.

Almonds have the characteristic lignified epidermis.

Pimenta has the diagnostic stone and oil cells.

Capsicum has characteristic stone cells and epidermal cells.

Staphisagria: The pored parenchyma and epidermis are diagnostic.

Foeniculum has five ribs and one oil tube between each rib.

Coriandrum has five primary, four secondary ribs, with one oil tube between each.

Carum has five ribs, six oil tubes, characteristic trichomes and no starch.

Anisum has five ribs and from twelve to twenty oil tubes.

Conium has five ribs but no oil tubes. Sections of Conium give inside one half hour, a mouse odor, when 25% NaOH solution is added. Old Umbelliferous fruits may give the same odor, when treated in this way.

Caryophyllus contains numerous oil cells, characteristic past and crystals.

Apii Fructus has twelve to sixteen oil tubes.

Petroselinum contains six oil tubes.

Piper is identified by the stone cells, hypoderm cells, needle shaped crystals of piperine, resin or oil cells and starch.

Cubeba is characterized by having secretion cells with a green content, characteristic stone cells and secretion cells, which turn crimson with sulphuric acid.

Aurantii Amari Cortex contains many cells with chromoplasts, many oil cells, and cells whose contents are yellow with KOH solutions.

Some of the Fruits and Seeds having:

Trichomes—are Anisum, Humulus, Strophanthus an Nux Vomica.

Characteristic stone—are Piper, Cubeba, Pimenta, Sabal Juniperis, Pepo, Coffee, Sinapis Alba and Nigra, Capsicum Linum and Carophyllus.

Characteristic secretion cells—are Sabal, Pimenta an Juniperis. All the Umbellifera contain yellow brown oil o secretion cells, except Conium.

Aggregate starch—are Cubeba, Piper and Cardamomum

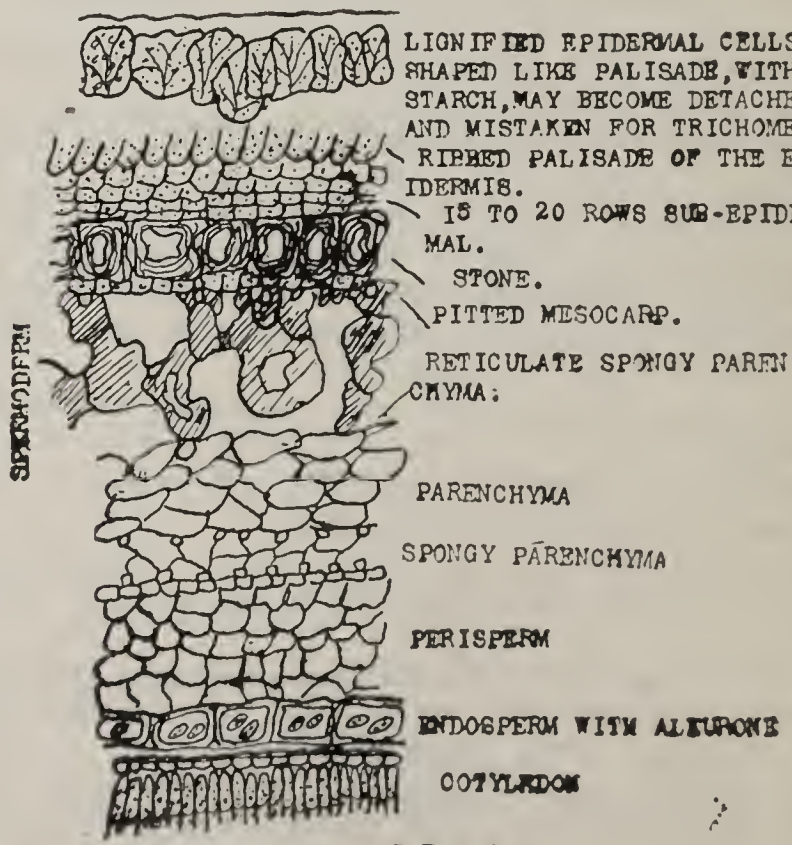
Characteristic starch—are Physostigma and Piper.

Characteristic mucilage and palisade cells—are the Mus tards.

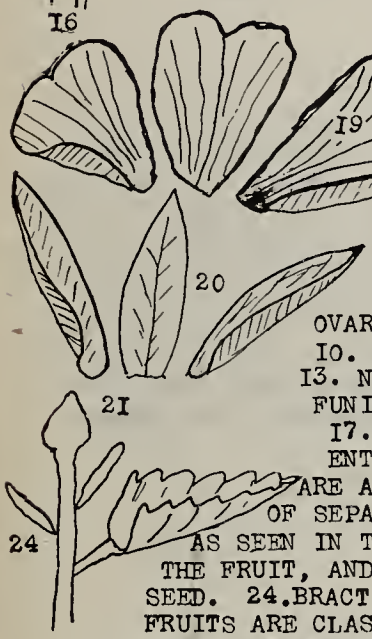
Characteristic thick walled cells—are Colchici Semen an Nux Vomica.

Characteristic Aleurone—are the Almonds, Linum, Pep and Strophanthus.

Epidermis with mucilage—are Linum and the Mustards



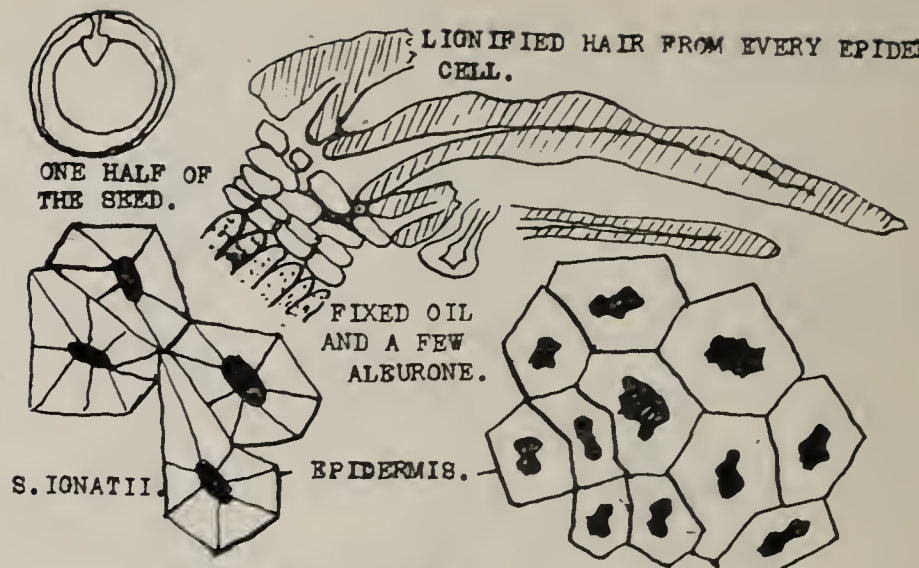
PEPO.



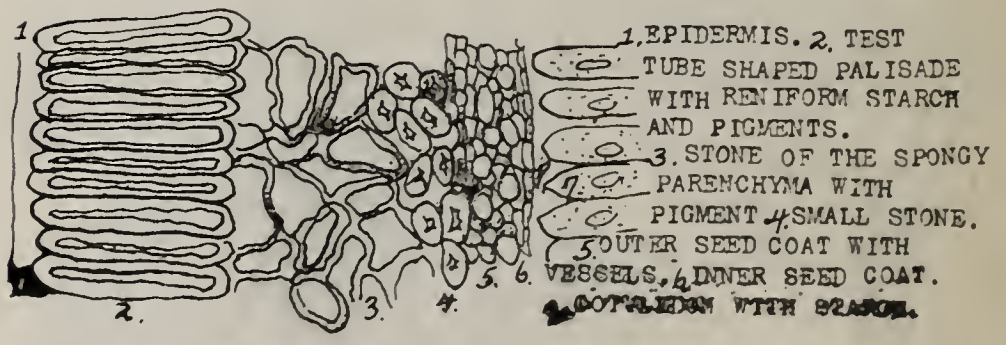
I. A PERFECT FLOWER. 2. TRANSVERSE VIEW. 3. GYNAECIUM AND ITS PARTS, 4. STIGMA, THE POLLEN RECEIVER, COMPOSED OF LOOSE TISSUE. 5. THE STYLE OR TUBE CONNECTING THE STIGMA WITH THE 6. OVARY, WHICH INCLOSES THE 7. OVULES, 8. LONGITUDINAL VIEW OF THE OVARY, 9. TRANSVERSE VIEW OF THE OVARY. I0. MICROPYLE, II. PRIMINE, I2. SECUNDINE, I3. NUCELLUS, I4. CHALZA, I5. PODOSPERM OR FUNICULUS. I6. ANDROECIUM, AND THE PARTS I7. ANTHER, THE POLLEN FORMER, I8. FILAMENT. I9. COROLLA COMPOSED OF PETALS WHICH ARE ANY COLOR BUT GREEN, 20. CALYX, COMPOSED OF SEPALS WHICH ARE GREEN, 2I. RECEPTACLE. AS SEEN IN THE APPLE 22. THE RIPENED OVARY BECOMES THE FRUIT, AND THE RIPENED OVULE 23. BECOMES THE SEED. 24. BRACT

FRUITS ARE CLASSIFIED AS INDEHISCENT AND DEHISCENT, DEHISCING ALONG THE VENTRAL SUTURE, THE DORSAL OR BOTH AND TRANSVERSE . THE FRUIT IS COMPOSED OF; PERICARP OR OUTER PART, WHICH IS COMPOSED OF THE EPICARP OR SKIN, THE MESOCARP OR EDIBLE PORTION AND THE ENDOCARP OR PUTAMEN OR SEED, THE SEED OR THE SPERMODERM IS COMPOSED OF THREE PARTS, THE TESTA (PRIMINE,) OR OUTER COAT, THE (SECUNDINE) TEGMA, THE INNER COAT AND THE (NUCELLUS) COTYLEDONS. CREMOCARP IS A DOUBLE FRUIT, EACH HALF BEING CALLED A MERRICARP.

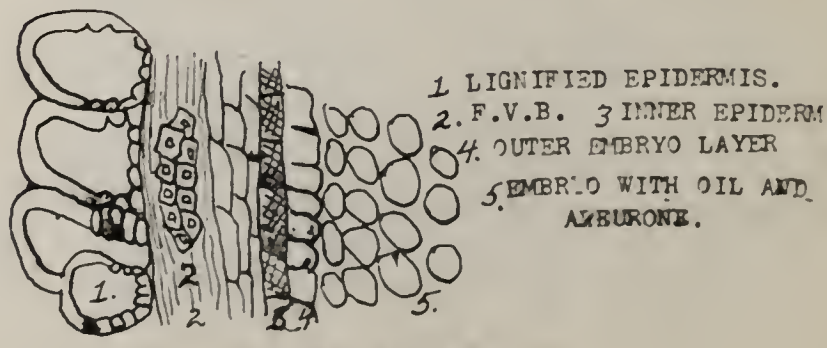
THE FORMATION OF FRUITS AND SEEDS FROM FLOWERS.



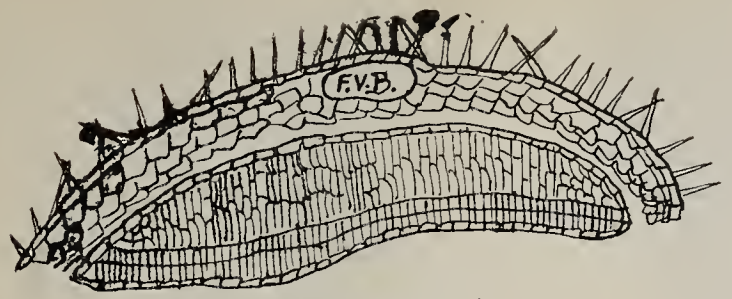
NUX VOMICA.


1. EPIDERMIS. 2. TEST TUBE SHAPED PALISADE WITH REN IFORM STARCH AND PIGMENTS.
3. STONE OF THE SPONGY PARENCHYMA WITH PIGMENT 4. SMALL STONE.
5. OUTER SEED COAT WITH VESSELS, 6. INNER SEED COAT.
7. COTYLEDON WITH STARCH.

PHYSOSTIGMA.

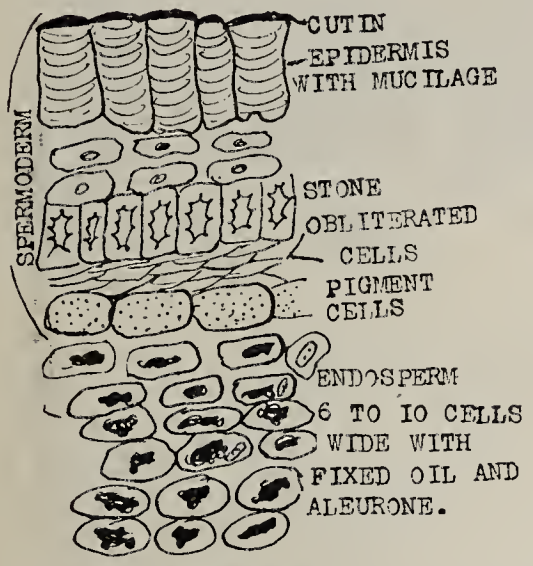


ALMOND.

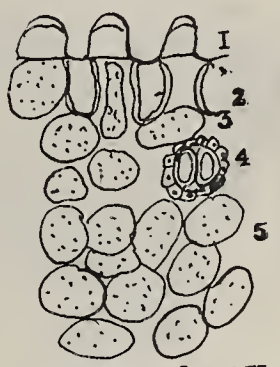


9 TO 30 ROWS POLYGONIAL CELLS.
SEVERAL ROWS COLLAPSED CELLS.
THE COTYLEDON WITH ALEURONE, FIXED OIL AND STROPHANTHIN, WHICH TURNS GREEN WITH H_2SO_4. 4. LIGNIFIED HAIR
ONE HALF OF THE SEED
SLIGHTLY

S T R O P H A N T H U S .

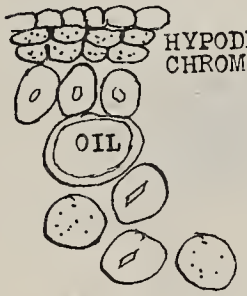


L I N U M .

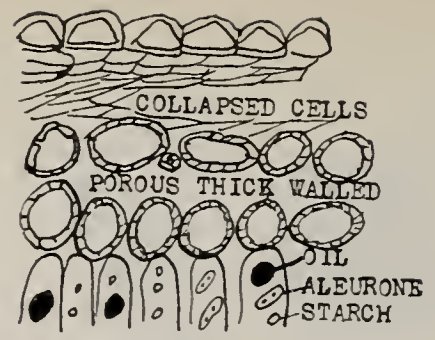


1. EPIDERMIS. 2. OIL.
3. CHLOROPHYLL CELLS.
4. FIBRO-VASCULAR BUNDLE.
5. CONTENTS YELLOW WITH KOH SOLUTIONS.

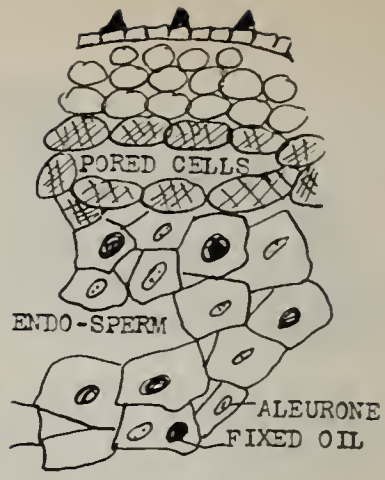
AURANTII AMARI CORTEX.

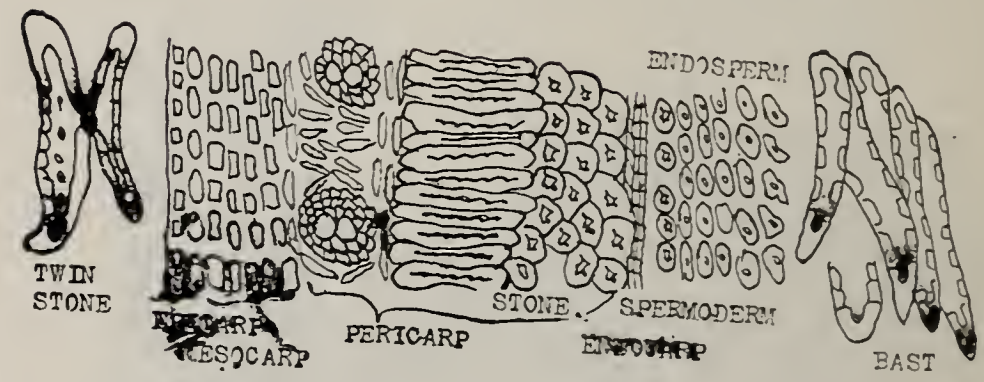


LIMONIS CORTEX.

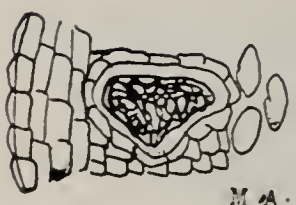


COLCHICI SEMEN



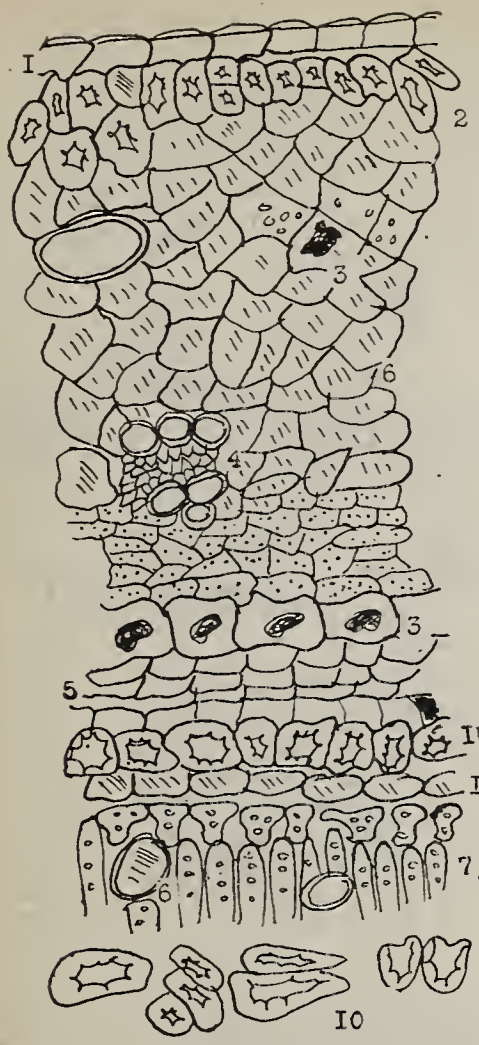


C O F F E E .



TRUE OR BANDOR MACE GIVES NO COLOR CHANGE WITH 10% KOH SOLUTION. WILD OR BAMBAY MACE GIVES A DEEP ORANGE COLOR.

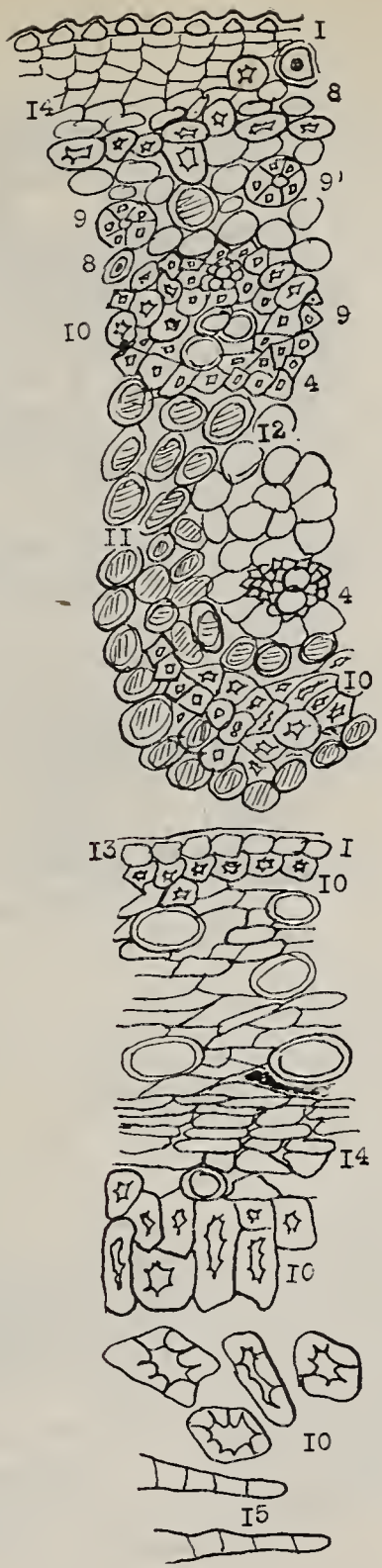
M A C I S .



I.EPIDERMIS. 2.HYPODERM.
3.OIL. 4. FIBRO-VASCULAR
BUNDLES. 5.ENDOCARP.6.
6.PIPERINE. 7.PALISADE
WITH STARCH AND ALEURONE.
8.SECRETION CELLS WITH GREEN
CONTENT. 9.BAST.I0.STONE.
II.LIGNIFIED POROUS CELLS.
I2. I0 ROWS SECRETION CELLS
WITH RESIN AND CRYSTALS,TURNS
CRIMSON WITH H_2SO_4 I3.PERISPERM

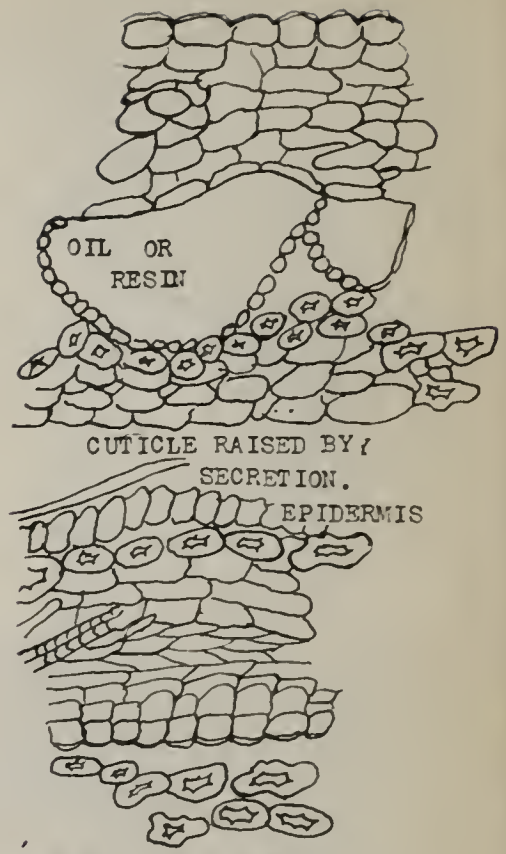
I5. HAIRS. I4.PARENCHYMA

P I P E R .

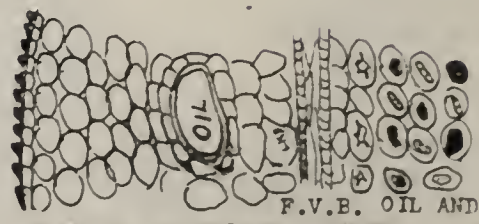


C U B E B A .

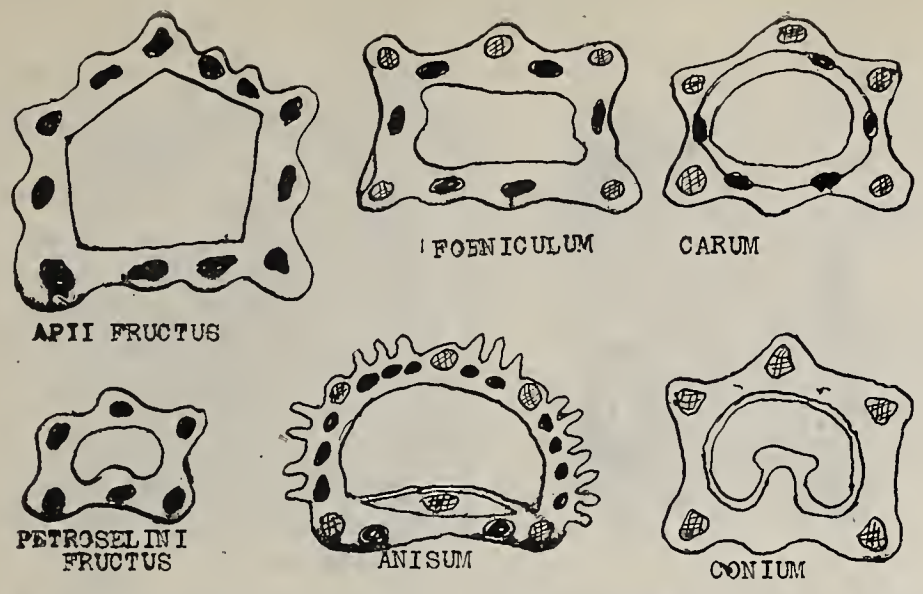
PIMENTA..



CAPSICUM.

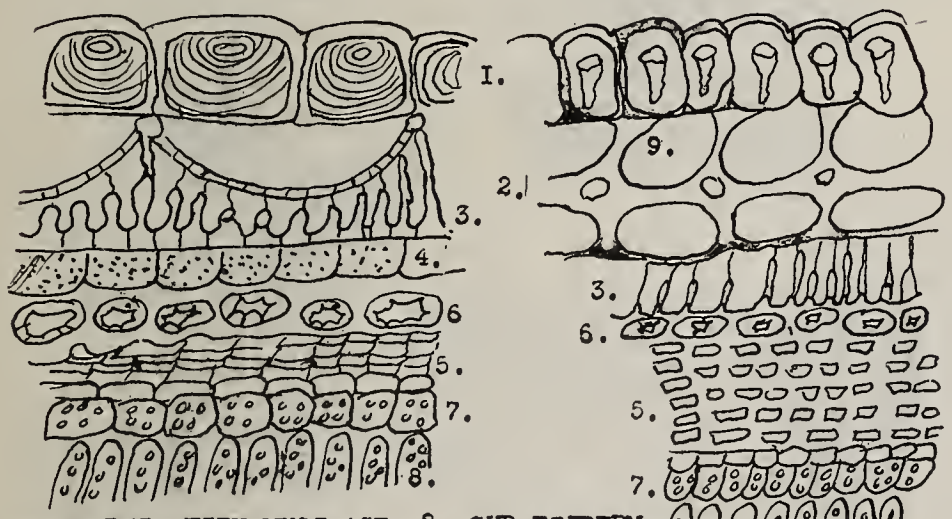


PETROCARYA NUTTS.



FOENICULUM HAS 5 FIBRO-VASCULAR BUNDLES,5 RIBS, 6 OIL TUBES.
CARUM, 5 RIBS,6 OIL TUBES AND NO STARCH.
ANISUM, 5 RIBS, I2 to 20 OIL TUBES,MANY NON GLANDULAR HAIRS.
CONIUM,THE ONLY CREMOCARP NOT FUSIFORM OR OBLONG,HAS 5 RIBS,AND NO OIL.
APII FRUCTUS HAS I2 to I6 OIL TUBES.
PETROSELINI FRUCTUS HAS 6 OIL TUBES.
CORIANDRUM,5 PRIMARY,4 SECONDARY RIBS AND 2 OIL TUBES.

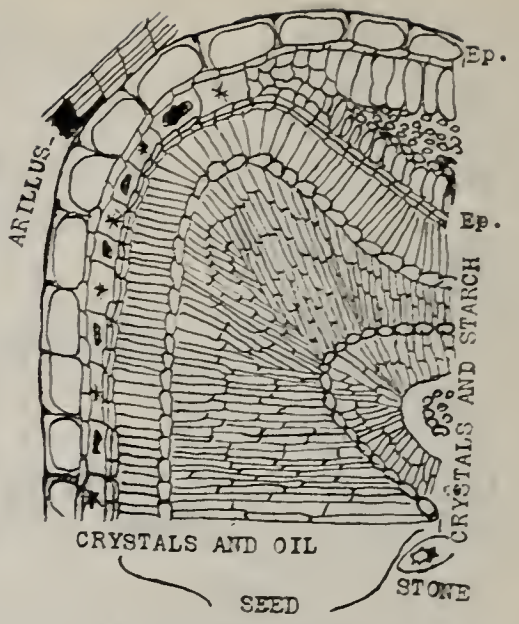
S O M E O F T H E C R E M O C A R P S .



I. EPIDERMIS WITH MUCILAGE. 2. SUB-EPIDERMIS, 3.PALISADE , 4.PIGMENT CELLS, 5. COLLAPSED CELLS, 6. ENDOSPERM, 7. COTYLEDON. 8 ALEURONE. 9.PARENCHYMA

S I N A P I S N I G R A . S I N A P I S A L B A .

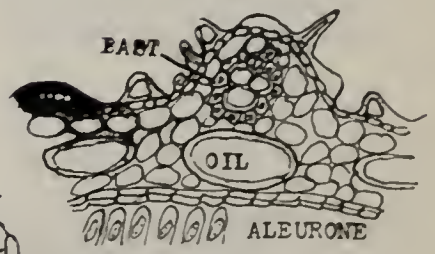
FOENICULUM.



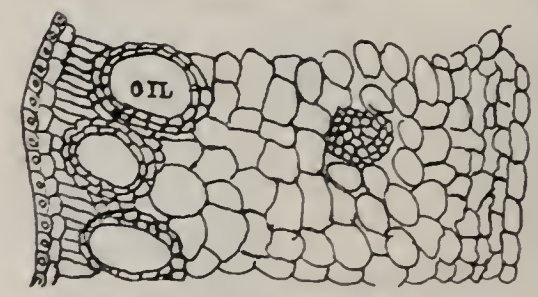
CARDAMOMUM.

CARUM.

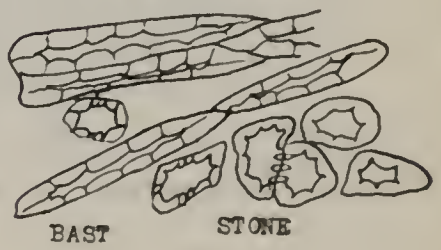
CORIANDRUM.



ANESUM.



CARYOPHYLLUS.



SOME DRUGS NOT INCLUDED IN PREVIOUS CLASSIFICATIONS

HONEY

Honey is a saccharine product formed through the transformation of the plant nectar by the honey bee. The nectar is supposed to be acted upon by certain salivary secretions of the bee and changed into sugars. Usually nectars of clover and buckwheat are the principle sources of the commercial article. Some nectars as those of Kamala and Rhododendrons act as a poison and emetic to man. Microscopically, honey contains crystals of cane sugar, pollen, wax and parts of the bee-wings, legs, etc. The source of the honey, if unstrained, may be determined by the pollen grains, which should be present, and no foreign matter of any kind should be present.

EXERCISE

1. Make a temporary mount by placing a small drop of the honey on a slide and covering with a cover glass. Report and make drawings of: (a) Name the pollen, (at least name the family) (b) Starch (in honey made by bees fed on meals and sugars in the early spring); (c) Ultramarine specks (from sugar); (d) Spores and fragments of fungi and bacteria (from careless preparation of the honey, rendering it unfit for use); (d) Insect fragments; (e) Sugar crystals; (f) Any other cellular forms present. Remember there is little or no pollen found in strained honey.

OPIUM

Opium in glycerin mounts appear as grayish brown, irregular masses or granules. There should be little or no starch present. Some thick walled polygonal cells from the capsule epidermis may be seen. Epidermal cells of the Rumex leaf which has been used to wrap the opium (Indian opium is paper wrapped) are polygonal on surface view, with many elliptical stomata, which have a narrow opening. Fragments of the Rumex fruit—used to prevent cohesion, are identified by the fibro-vascular tissue with spiral tracheae and narrow sclerenchymatous fibers. The parenchyma of the poppy seeds is light in color and contains air. The epidermal cells with oblique pores are diagnostic. Smyrna opium usually contains the greatest number of epidermal cells. Persian opium contains much starch and few epidermal cells—sometimes fragments of paper, from the wrapping. Egypt-

ian opium contains poppy leaf fragments, as also Turkey opium, which contains in addition fragments of the red poppy flowers.

EXERCISE

1. Make a temporary mount in chloral T. S. and examine for the yellow-brown or brownish, irregular fragments of the latex, poppy capsule, leaf cells, and the rumex fruit cells. Draw.

2. Stain another mount with ligno-cellulose stain and examine for the lignified thick walled 4 to 5 sided, or narrow elongated poppy capsule epidermal cells, and fragments of the tracheae of the leaves.

3. Test another mount for starch.

4. Make a permanent mount of an untreated specimen.

VANILLA

Vanilla.—Make transverse and longitudinal sections. Note: The epicarp is composed of thick walled, finely porous cells, with longitudinal rows of stomata, elliptical or more often circular. The cuticle is thin and yellowish brown. Imbedded in the ground substance are brownish bodies, prisms of calcium oxalate and vanillin. The hypoderm cells have thick walls—large beaded walled collenchyma. Mexican, Panama, Honduras and Central America varieties have characteristic, spirally elongated pores. These are not found in the Bourbon, or the common varieties. Here the pores are rounded or oval. The parenchyma of the pericarp is loose and end to end, in longitudinal rows, and contains raphides of calcium oxalate and vanillin, (test for vanillin—add a solution of phloroglucin, then sulphuric acid, a carmine color results,—Distinction from Benzoic Acid). The fibro-vascular bundles are collateral, with spiral, or reticulate ducts, joined porous elements, sieve, bast. The bast have oval pores and many have fissures. The inner epidermis is located between the three pairs of placenta bars, and contains many thin walled glandular papillae, filled with balsam. The epidermis of the placenta has thin walls and elongated cells. On the surfaces between the placentae the epidermal and sub-epidermal are made up of large bundles of thread like mucilage cells, used for the conduction of pollen. The spermoderm contains the minute black seeds, which owing to their black pigment, must be boiled with KOH solutions before being crushed for structure examinations. The outer epidermis is polygonal in shape, and the cavities contain narrow slits.

The inner epidermal layers are elongated, and the parenchyma is elongated and dark brown. The endosperm being absent, the kernel of the seed consists entirely of embryo, usually undeveloped.

SAFFRON OR CROCUS

Place several of the dried stigmas in water for three minutes, transfer to 80% ammonia water for three minutes, then wash the stigmas with water and mount in chloral T. S. and water equal parts. The stigmas show an epidermis consisting of long narrow cells with delicate wavy walls; some of these cells have small curved papillae. The epidermal cells at the stigma apex have very large papillae, to which very often pollen is seen adhering. Several layers of elongated parenchyma cells are seen below the epidermis and near the center of the stigma are very small fibro-vascular bundles. Scattered through the interior of the stigma are many secretion tubes. When C. Z. I. is added to the water and chloral mount, the cell walls turn blue and the cuticle, which often separates, turns yellow. When the stigmas are mounted in chloral T. S. and iodine, starch grains are seen in the pollen grains. When the powder is added to a drop of C. P. sulphuric acid, each particle is surrounded by a blue liquid, rapidly changing to violet and then brown.

Weighted saffron.—Saffron to which magnesium sulphate has been added. Test: Place stigmas on slide, moisten with chloral test solution and cover. Fine needle (short or long), prisms, stellate, sheaf or tufts appear. These are insoluble crystals will appear. A solution of calcium chloride added in alcohol. Then add a drop of sodium phosphate solution and expose to ammonia vapors, the characteristic snow flake to weighted-saffron stigmas, develops the characteristic gypsum crystals. With the adulterants no blue color changing to voilet, then to wine red; but where you add 60% sulphuric acid in the case of pure or true saffron, this color appears. A 5 to 10% solution of sodium phosphomolybdate gives a more permanent blue, then adding to the specimen any mineral acid (5 to 10%) a green color results in the true saffron. With this test the adulterants and coloring substances as safflower, curcuma, paprika, zea, sandal wood, log wood, coal tar dyes, carmine and cochineal give other colors than green. On adding 60% sulphuric acid to saffron, a blue color, changing to violet, then to wine red, is produced. This color reaction will not take place with the adulterants.

CALENDULA

Calendula florets mounted in water or chloral T. S. show elongated epidermal cells with a striated cuticle. The underlying parenchyma cells contain many oil globules. Characteristic multi-cellular hairs are seen near the cololla tube

SAFFRON AND CALENDULA
EXERCISE

1. Mount part of the flower in water. Draw.
2. Irrigate with ammonia water, several times; then transfer to 80% alcohol, make drawings.
3. Mount another specimen in chloral T. S. 8 parts and water 5 parts. Make drawings.
4. Stain with C. Z. I. and make a permanent mount. Draw.
5. To another specimen add sulphuric acid. With saffron a deep blue color results, changing to violet, then to brown. Set the slide away for 20 minutes and then examine for thin needle shaped crystals in the crumpled cell masses: these crystals are insoluble in water, or HC1, and are not calcium compounds.

CACAO

When water mounts of cocoa are heated to the boiling point, many oil globules separate out. The starch is very small and hard to gelatinize; the epidermal cells of the cotyledon are red-brown. If powdered cocoa is boiled in chloral T. S. the fragments become clearer and the following cells may be seen: The thin walled parenchyma of the cotyledons, minute starch grains, simple and rounded, or composed of two or three grains, the polygonal epidermal cells of the cotyledons, containing a dark red-brown content, a few characteristic trichomes. many fat globules, and cells containing the red-brown cocoa-red, which is soluble in alkalies.

EXERCISE

1. Make a temporary mount. Draw all the elements. Then add sulphuric acid, which turns the dark brown cells a crimson red.
2. Warm another temporary mount, causing the fat to separate out and the very small starch grains hard to gelatinize. Look for the fragments of the red-brown cotyledon and endosperm epidermal cells. Stain with Tr. Alkanet.
3. Defat another specimen with ether, alcohol or 10% KOH. Then mount in water and add chloral T. S.. then

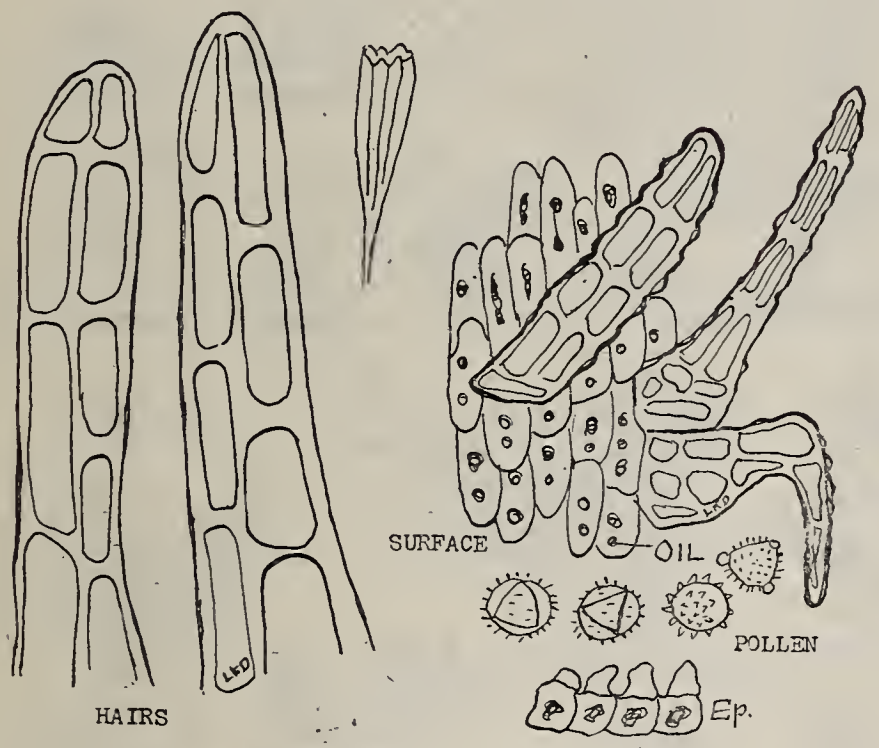
iodine T. S. Draw. Then warm the slide. The elements will become clearer. Draw.

Look for the delicate colorless cells of the cotyledon, calcium oxalate, fragments of the fibro-vascular bundles and the characteristic starch and trichomes.

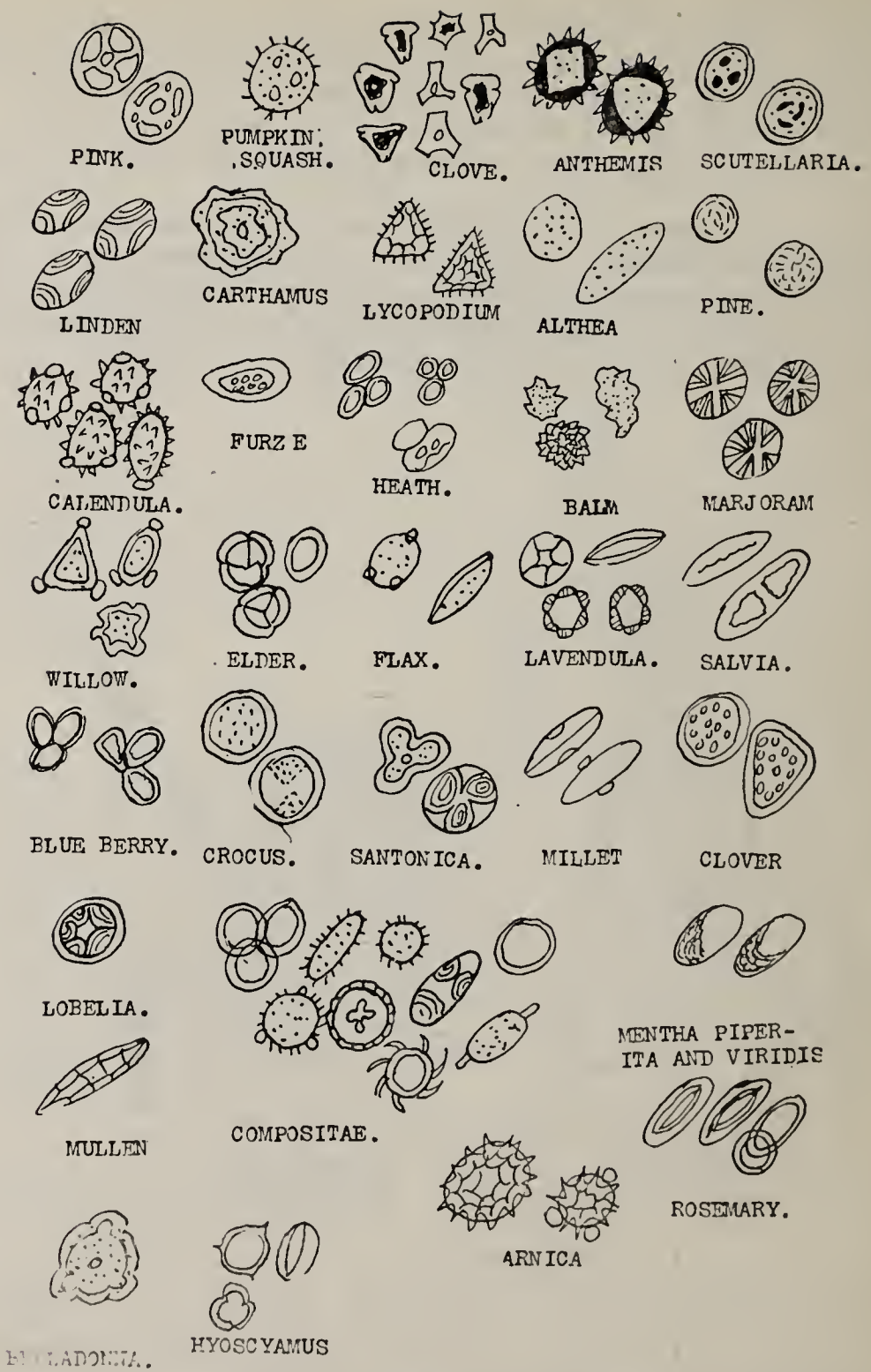
Make a permanent mount of an untreated specimen.

4. Adulterants present, mineral matter, foreign cells, etc. etc.

Diagnostic points.—(a) Thin walled parenchyma of the cotyledon; (b) Minute starch, 2 to 3 compound, very hard and slow to gelatinize; (c) Polygonal epidermal cells of the cotyledon containing red-brown granules; (d) Trichomes; (e) Cells containing coloring, or cacao red.

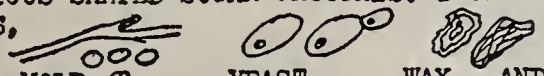


CALENDULA.



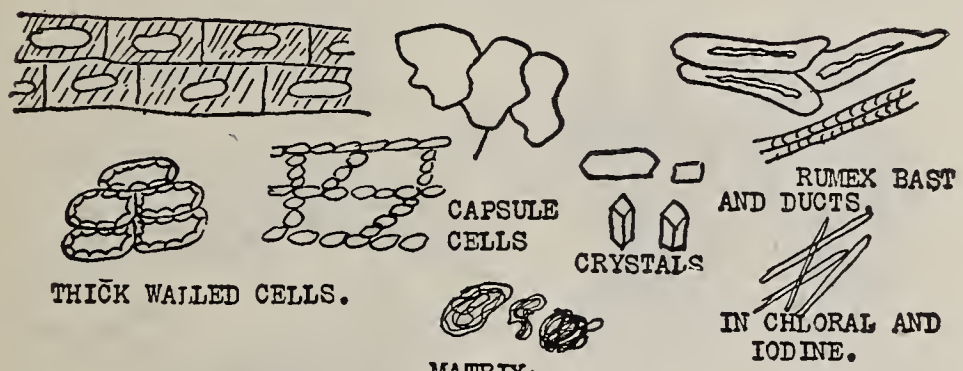
POLLEN FROM VARIOUS PLANTS.

VARIOUS SHAPED SUGAR CRYSTALS. INSECT PARTS AS FEET, WINGS,



MOLD. YEAST. WAX AND VARIOUS POLLENS.

H O N E Y .

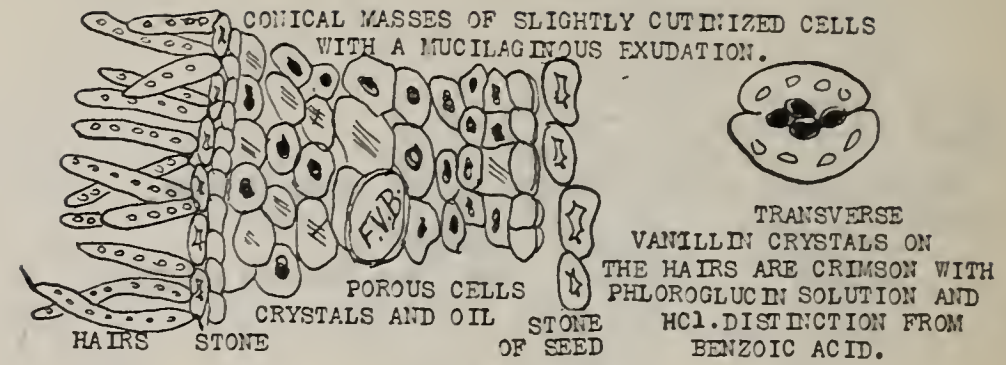


IN THE UNRIPE CAPSULE, THE EPIDERMAL CELLS ARE PARTLY SILICIFIED, IN THE RIPE CAPSULE THE EPIDERMAL AND UNDERLYING TISSUE ARE SILICIFIED.

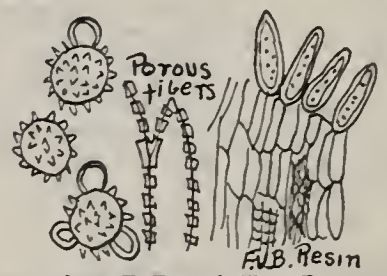
O P I U M .

MUCUNA.
(COWHAGE)

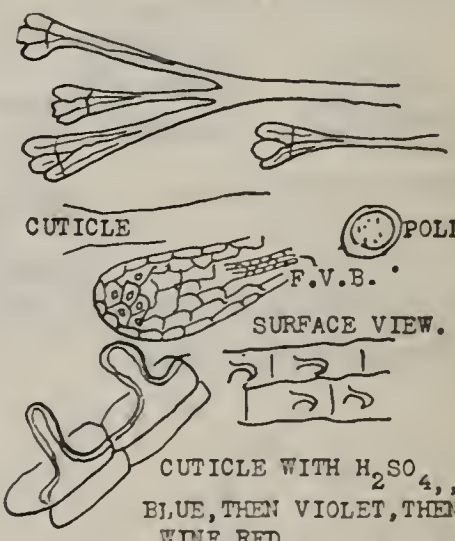
ARTEMISIA ABSINTHIUM


CONICAL MASSES OF SLIGHTLY CUTINIZED CELLS WITH A MUCILAGINOUS EXUDATION.
F.V.B.
TRANSVERSE
VANILLIN CRYSTALS ON THE HAIRS ARE CRIMSON WITH PHLOROGLUCIN SOLUTION AND HCl. DISTINCTION FROM BENZOIC ACID.
POROUS CELLS
CRYSTALS AND OIL
HAIRS
STONE
STONE OF SEED

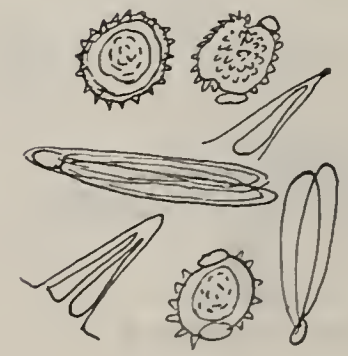
V A N I L L A .



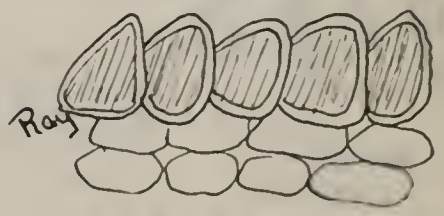
S A F F L O W E R.
(CARTHAMUS TINCTORIA)



C R O C U S .
(SPANISH SAFFRON)

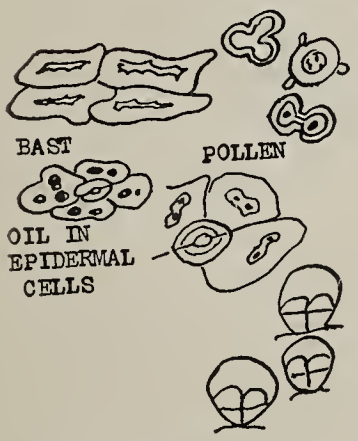


C A R T H A M U S .
(AMERICAN SAFFRON)

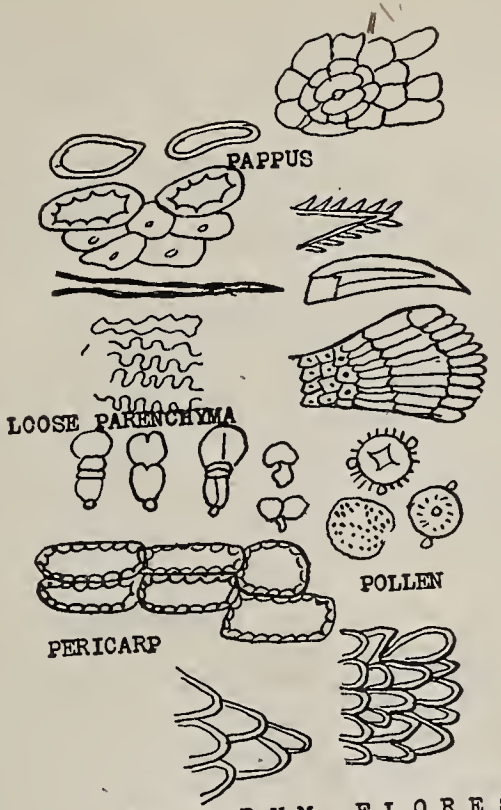


A N T H E M I S .

CUSSO.

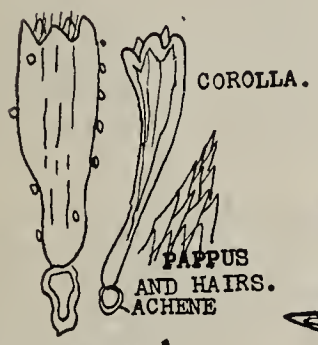


SANTONICA.



PYRETHRUM FLORES.

RHUS GLABRA.



ARNICA (FLORES.)

LAVENDULAE VERA

Draw sections of an official leaf, name the leaf and all the cells. Do this before the next period.

THE NATURAL POWDERS

Powders may be divided into two kinds.

1. Artificial.—Those obtained by grinding, rasping or crushing, as powdered drugs.

2. Natural.—Those occurring as such and obtained without treatment, as Lupulinum, Lycopodium, Kamala, Terra Silicea Purificatum.

Natural powders may be classified as:

1. Cell-like forms, as glands—Kamala and Lupulinum.

2. Cell contents—obtained by special processes as Amylum.

Every natural powder has its own peculiar form, revealed by the microscope. In the study of these forms, such points as the following must be noted: Size, uniformity of size, color, shape, tendency to cohere in masses, or remaining separate, reactions to reagents, inflammability, solubility, ash, odor on heating, affinity for acid or alkali stains. Beaker sand test—Powder placed in a beaker of water, sand sinks to the bottom.

LYCOPODIUM

The spores are low, broadly triangular pyramids resting on a convex base. They have one convex surface and three plane surfaces. The line of union of these surfaces may appear as a cross or triangle. Their shape is produced by the pressure of the spore in the cell in which they are formed. The convex base is covered with a delicate network. It extends over the flat sides and nearly to the angles of the sides. The network consists of raised colorless transparent ridges, appearing as reticulate markings on the surface of the spores and projecting over the edges, forming the saw tooth like appearance. Between the teeth the membrane is stretched which forms the ridges, when the spore is seen lengthwise.

EXERCISE

1. Make a temporary mount. Draw 3 or 4 spores, each lying in different positions, so that all the sides may be seen. Draw the outline first, then fill in the reticulations and count them. Represent the shape accurately. Focus to see all parts clearly.

2. To the temporary mount, press the cover glass so that the spores will be crushed. They usually burst along the angles and the contents are discharged, which is a liquid, consisting of protoplasm and fixed oil in globules. Note:

(a) Globules, which are regular and uniform, bounded

by a narrow dark line and a very bright center are air globules. No broad black border.

(b) Irrigate the slide with Tr. Alkanet, diulted with an equal part of water, the oil globules will stain reddish.

3. To another slide prepared as in No. 2, add a 1 per cent solution of Osmic Acid (use care to keep this solution from the hands—it must be kept in the dark, and will not react with all fixed oils, or fats as palmatin, stearin, etc.) The oil globules stain brown black with Osmic Acid.

4. Test another specimen for starch.

5. Draw any foreign pollen which may be present.

6. Mount in chloral T. S. Draw.

7. Make a permanent mount.

LUPULINUM

The glands under low power appear as a single hemispherical layer of cells, the common cuticle covering them has been raised dome like by the secretions of oil. Under high power the cuticle is seen to have the impression of the cell wall on which it originally rested. The glandular trichomes as seen from the tops are nearly circular and the outer layer of cells are in focus, while the lower curved part, is indistinct. From the side they appear mushroom shaped.

EXERCISE

1. Make a temporary mount by placing the glands on a slide; add one drop of alcohol and immediately one drop of glycerine. If the alcohol is permitted to remain too long, it will dissolve the oily contents. Lower the cover glass carefully so that the glandular trichomes will not be crushed. If there is any grittiness felt when the cover glass is rubbed over the slide, brick dust or sand may be suspected as an adulterant. Make drawings.

2. Carry out exercises No. 2 as given under Lycopodium.

3. Carry out exercise No. 3 as given under Lycopodium.

4. To a temporary mount, place a hair under one edge of the cover glass to prevent crushing; then irrigate with 10% KOH; immediately place under the microscope and see the oil dissolving out.

5. Mount in chloral T. S. and examine for vegetable debris.

6. Make a permanent mount.

KAMALA

Kamala is a fine granular mobile, dull red powder, con-

sting of the trichomes and glandular trichomes of the fruit f Mallotus philippenesisi, Euphorbiaceae. It is composed of roups of trichomes and small garnet red glands. Each roup of trichomes consists of a tuft of thick walled, short ointed, diverging trichomes, sometimes containing air, or a ranular substance, or a reddish resinous substance. The richomes may be divided by a delicate transverse wall into , 3 or more cells. Note that the hairs are rarely single. 'he glandular trichomes are so deep in color that they must e cleared before the structure can be seen. When cleared hey appear as consisting of a number of elongated cones ra- iating from a common center and enlarged at the free ex- remities. They are enclosed by a delicate membrane, which s the original cuticle covering the secreting cell. The space etween this cuticle and the cell contains resin. Secretions ake place on the radial and outer walls.

EXERCISE

Moisten a teasing needle in water, dip the needle in the owder and transfer the adhering particles to a drop of water n the center of a clean slide, cover, fill the space between he cover and slide with water, if necessary; move cover over slide so that the powder may be uniformly spread; in doing so you may feel grit or grating due to adulterants as brick dust, sand, etc. Examine with the low power, thein use the high power. Note points given above; also if there are amorphous bodies, crystals, bodies made up of concentric layers, fragments of leaves or other debris, any form dissolving in water, splintery fragments, opaque bodies, et al. Probably the most common and harmless of all adulterants are starches and dextrin. Any rounded or irregular shaped bodies marked by a hilum or lamella may be starch or dextrin; try the iodine test. If no starch like body is found, heat the specimen, to expel the air and cause the specimen to swell. If the specimen is not affected, use KOH solutions. In making a report, it is usually sufficient to say: The drug is adulterated. No one man knows the histological structures of every plant, but we can at least say from what plant part the adulterant has been taken.

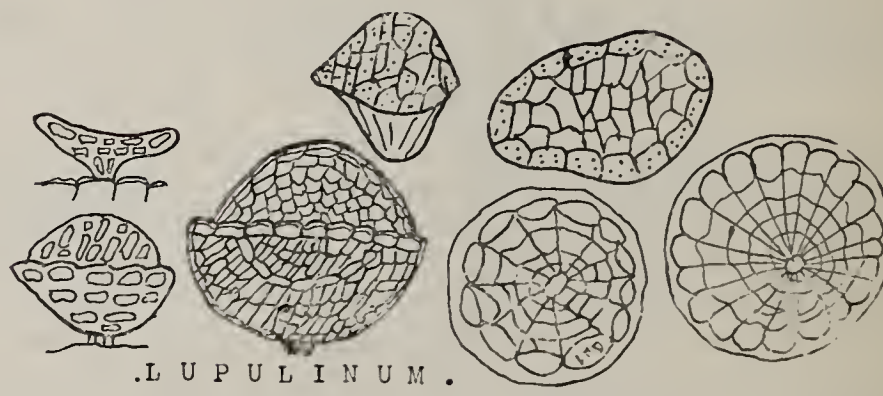
Adulterants may be divided into two kinds:

1. Intentional—as sand, dirt, brick, dust, chalk, saw dust, et al. It could hardly be other than intentional, when found in powders.

2. Accidental—as in Lupulinum; if it contains a few leaf cells, but only a certain number should be permitted.

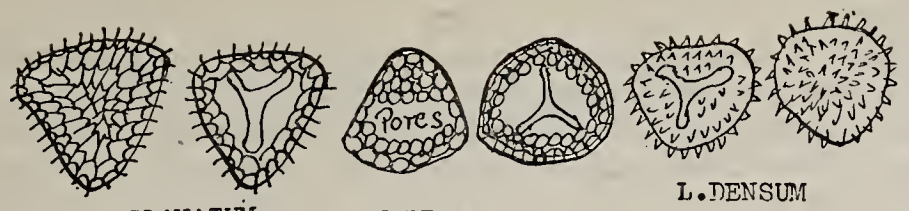
Sometimes fragments of the stem tissue, where leaves or roots are used, may be found.

Mineral matters are usually crystalline, transparent, as Talc, or opaque, as Sulphur—all are usually insoluble in water, alcohol or chloroform. Organic masses, when opaque and irregular in outline, may be suspected of being resin (which may be saponified with KOH), and are soluble in alcohol, ether or chloroform. Brick dust usually sinks when stirred in water or chloroform. Have you cleaned your microscope?

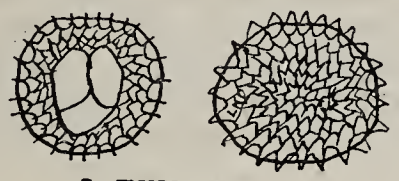


.LUPULINUM.

KAMALA.



L.CLAVATUM L.PHYLLANTHUM L.DENSUM



L.INUNDATUM

LYCOPODIUM

ARTIFICIAL AND NATURAL FOODS

As there is little, or no ptyalin in the babe's saliva until at least the second month, (most authorities claim 12 months), starches cannot be digested before this time, unless the starch has been modified.

Starch may be digested to the extent of a small per cent after the fourth month, if it is modified.

There are many infant or invalid foods on the market and nearly all are modifications of the mother's milk,—fats 3., lactose 5., proteins 0.75 and alkali water qs. (lime water 0.5% is usually added for the alkali.) Some artificial foods contain egg albumen for the proteins. Milk-sugar must first become dextrose before being absorbed. Codliver oil, or yolk of eggs are absorbed as emulsions. All the constituents of the artificial foods should be in a perfect emulsion for rapid absorption.

Artificial foods do not take the same place as mother's milk for the babe. This was shown during the cotton famine of 1880. When the women workers were idle and nursed their babes, the mortality decreased over 50%. During the siege of Paris in 1870-71, the supply of cow's milk being shut off, the mothers were compelled to nurse their babes, and the mortaliy dropped from 33% to less than 7%.

The powdered foods are desiccated and usually lack fat, which may be added in the form of cream, or oils as olive or cotton seed.

Many of the foods contain unconverted starch, which is not digested in the stomach of the babe.

The following are necessary for building up the body: Fat is used for building new cells, and along with the sugars, produces force and heat. Heat is necessary for digestion. Proteins increase the oxidation of the tissues and are also used as building material. Salt furnishes the gastric juice with HCl. These must be combined in the proper proportions and in such a way that a perfect emulsion is formed for good assimilation. Few foods on the market conform to these requirements.

The average composition of milks:

	Mother's	Cow's	Goat's
Fat	3.78	3.64	4.78
Casein	1.03	3.02	3.20
Albumen	1.26	0.53	1.09
Lactose	6.21	4.88	4.46
Water	87.41	87.22	85.71
Total solids	2.59	12.78	14.29
Ash	0.31	0.71	0.76

Ayrshire cows give the most lactose, 5.33%; Jersey cows give the most fat, 5.61%, and also the highest total solids, 15.41%; Devon cows give the most casein and albumen, 3.76%, and also the most ash, 0.76%.

Cow's milk forms a solid curd in the stomach and therefore is less digestible than mother's milk, which gives a soft flocculent curd; so that barley water and other cereal waters are added, to help to divide the curd and to aid the action of the gastric juice. They also aid in preventing fermentation.

While much may be said for or against the various foods, nearly all agree to the following:

1. The food should be finely divided. 2. Uniform. 3. It should contain the proper elements and in their proper proportion—fat, sugar, protein and alkali. 4. It should be free from all micro-organisms. 5. It should have no unconverted starch. 6. It should be in such a condition as to form a perfect emulsion. Few foods answer all these requirements.

Microscopically:

Fat is seen in uniform globules, staining red with Tr. Alkanet, or black with Osmic Acid. Proteins are in irregular masses and stain brown with iodine solutions. Sugar occurs in typical crystals soluble in water; and also salt, which

occurs as cubical crystals. Dextrin resembles somewhat in outline, starch, staining yellow with iodine solutions, and is soluble in water.

The absence of fat is partly responsible for rickets—faulty bone growth or the lack of nourishment to growing cells may produce this disease. It may also be caused by too much sugar, or the continued use of certain condensed milks.

Too much sugar or food, or air in the bottle may produce colic.

Goat's milk is the nearest to mother's milk in digestibility and much easier to digest than cow's milk.

Cow's milk is the universal substitute. And as the cow is very prone to disease, such as consumption, many cases of sickness have been traced to the cow, such as consumption of the intestines, and streptococcus infections coming from sore udders etc.

Wet-nursing is especially dangerous on account of disease being communicated and is going "out of style".

Nipples should be constructed so that they may be easily turned inside out for thorough scrubbing. They should be scrubbed after every using, rinsed in hot water and placed in a covered glass with borax water, until again used. After being washed that part which goes into the babe's mouth should not be touched by the hands, for the hands always contain micro-organisms. By dropping a little of the warmed milk on the wrist one may readily determine if it is too warm or too cold. The nipple should never be placed in the nurse's mouth, to determine the temperature of the milk.

Bottles should be boiled after each using. They should be constructed to afford ready cleaning—no shoulders, small necks or corners; for the brush cannot clean all the milk out of these places; and as milk is typical food not alone for the human but for many micro-organisms, these flourish, and in many cases of improper cleaning of bottles cause disease.

The energy value of a food is expressed in "calories". A calorie is the unit of heat measurement and means that amount of heat which is required to raise the temperature of one gram of water through one degree Centigrade.

Hence the caloric value of one gram of any food substance may be expressed in calorie factors as proteins—4.1 calories, carbohydrates—4.1 and fat 9.3 calories. But this value does not mean nutrient value as not all the food is utilized; and some of the calories are utilized in converting food so that it may be assimilated.

Draw sections of a typical rhizome, name the cells. Show to the instructor for credit. Do this before the next period.

In health, the normal diet in 24 hours contains—proteins 20 grams, 492 calories, carbohydrates—500, or 2050 calories, fat—50, or 465 calories, a total of 3007 calories. If person is in bed, as in sickness, the amount of protein is decreased, on account of less muscular work and less material for repair being used; so the excess proteins are excreted as they cannot be stored for future use. The patient in this condition requires about 1800 or 2000 calories. The digestion of the proteins is complicated and requires tissue loss and energy; while the digestion of fats and carbohydrates is simple leaving only a little residue, and the part not used at once can be stored for future use. When these are used in excess, they prevent some of the proteins from being digested and made available for tissue repair.

To determine the amount of protein, carbohydrates and fat in a given amount of food—reduce the given quantity of food material to grams. Then multiply this by the per cent of proteins, carbohydrates and fats in that particular food material, (see table) as 1 gram of bread contains 6.5% proteins, and 51.5% carbohydrates and 1.5% fat; then 128 grams of bread times 6.5% proteins equals 8.32 grams of proteins, and 128 times 51.5% carbohydrates equals 65.92 grams of carbohydrates, and 128, times 1.5% fats equals 1.9 grams of fat.

To determine the caloric value of this given quantity of food material, multiply the actual amount of protein, carbohydrate and fat by the appropriate caloric factor, as 8.32 grams times 4.1 (protein caloric factor) equals 34.11 calories.
65.92 grams times 4.1 (carbohydrate caloric factor) equals ..... 270.27 calories.
1.9 grams times 9.3 (fat caloric factor) equals 17.67 calories.

The caloric value in 128 grams of bread is 322.05 calories.

Percentage of constitutents absorbed from mixed diets of animal food are protein 98%, fat 97%, carbohydrates 100%.

Percentage of nutrient constituents absorbed from cereals and sugars are protein 85%, fat 90%, carbohydrates 98%.

Percentage of nutrient constituents absorbed from:

	Porteins	Fats	Carbohydrates
Mixed diets of animal food	98	97	100
Cereals and sugar	85	90	98
Vegetables and fruits	80	90	98
Ordinary mixed diet	92	94	98

Calories available for nutrition from—Cow's milk 89%, mixed diet 89%, bread 82%, potato 92%, meat 76%.

The per cent of dry substances not absorbed—Wheat bread 4, macaroni 4, rice 4, meats 4½, eggs 4½, corn 7, peas 9, potato 15, turnips 20.

Vegetables can take up fat for each 100 parts as follows: Potato puree 50 parts, boiled or baked potatoes 40 to 50 parts, cabbage 40 parts.

The average waste in green vegetables of nutritive constituents is as follows: Proteins 18% lost, carbohydrates 15% and mineral 23%.

Fruits contain available sugars as follows: Grapes 16%, figs 11%, cherries 10%, canned pears 8%, strawberries 6%, currants 6%, raspberries 7%, oranges 8%, pineapples 13%, plums 2%, and lemons 1%.

There is a relative tendency of sugars to undergo fermentation and changes as follows: Lactic fermentation reults from levulose, lactose, dextrose, saccharose and maltose. Butyric fermentation results from maltose, dextrose, saccharose and lactose. Alcoholic fermentation results from maltose, saccharose, levulose and lactose.

The sugar assimilation limits are lactose 120 grams per day, saccharose 150 to 200, levulose 200, and dextrose 200 to 250.

Acid foods are oats, barley, wheat, eggs, rice and corn. Beef is also classed as an acid.

Neutral foods—sugars, vegetable oils, animal fats.

Alkaline foods—carrot, turnip, potato, onion, beans, lemon and orange juices. Milk is also classified as alkaline.

Food rich in purins—sweet bread, liver, beef, pork, mutton, chicken, veal and many fish.

Food poor in purins—potato, onions, oatmeal, turnips carrots, parsnips, asparagus, rhubarb, spinach, dates, figs, codfish, tea, coffee, cocoa, malt liquors.

Food containing no purins—milk, eggs, cheese, butter, sugars, white bread, rice, tapioca, cabbage, lettuce, macaroni, strawberries, wines and spirits.

These foods contain to each 1,000 parts the following amount of salt: bread 8 to 10, sea fish 5, milk 1.5 to 2.5, eggs 1.6, lentils 2.3, fresh butter 1, peas 0.6, meat 0.3 to 1, potato 0.5, fresh water fish 0.48, fruit 0.03, rice 0.02.

Minerals are required per day in foods as follows: Phosphoric acid 3 to 4 grams, sulphuric acid 2 to 3, potassium oxid 2 to 3, sodium 4 to 6, calcium oxid 0.7 to 1, magnesium 0.3 to 0.5 chlorine 6 to 8, iron 0.006 to 0.012.

Phosphoric acid is found in carrots to the extent of 0.036%, in turnips 0.058%, cabbage 0.089%, potatoes 0.14%, pork 0.16%, chestnuts 0.2%, milk 0.22%, barley meal 0.23%, beef 0.28%, eggs 0.33%, cheese 0.37%, mutton 0.42%.

Animal foods are rich in sodium, while vegetable foods are rich in potassium.

Foods containing much iron are spinach, yolk of egg, beef, apples, lentils, white beans, peans, potatoes, wheat and oatmeal.

Meat, fish, bread, fruit and potatoes contain very little calcium salts; while rhubarb, asparagus, spinach, rice, cereals and radishes contain considerable salts. Milk contains about 0.095 grams to the liter. An infant requires about 0.325 grams daily.

Sulphur is found in dried peas 0.4%, glutin 0.7%, wheat albumen 1.5%, dried white of egg 1.8%.

A cup of tea contains about 0.065 grams of caffein and about 0.215 grams of tannic acid. Coffee contains about 0.075 grams of caffein and about 0.216 grams of tannic acid. Cocoa contains about 4% nitrogenous matter, 20% fat, and 40% non-nitrogenous matter. Chocolate contains about 4% nitrogenous matter and 27.5% fat.

The intestinal flora is influenced greatly by diet. Lactose when fed in sufficient quantities brings about a complete change in two to three days, while milk requires a longer time and does not make a complete change. Milk and lactose form the most practical and effective diet for man. Grain tends to increase the number of aciduric micro-organisms, but their influence is little. Milk owes its beneficial action to the lactose, which constitutes almost half of the solid matter present for lactose is absorbed slowly from the intestine.

The raw grains are slowly acted upon by the intestine, or perhaps some of the intermediate carbohydrate products are not immediately absorbed.

Bread which contains partly cooked starch will not hasten the growth of the aciduric organisms, on account of its quick digestibility, and no available sugar remains in the intestine sufficiently long enough for the organisms to utilize it.

Meat, or other high protein diets, increase the indol producing organisms and other organisms of the so-called putrefactive type. Corn starch aids in the growth of the amylolytic group of intestinal organisms, while in a few cases grains seem to aid in the development of a fusiform bacillus

The reactions of the intestines in a number of experiments remained independent of the character of the intestinal flora. The acidity was highest in the duodenum and lowest at the ileoceal valve.

The number of calories required varies according to the age and occupation; as a child at birth needs per day 48 calories, and at the age of six to twelve months it needs 60 calories per day per Kg. weight.

Severe crying increases metabolism 40% per hour, the mechanical work of 4 hours crying would equal 29 calories.

Athletic boys—15 years of age, require 5,000 calories per day.

A soldier 35 years of age, 5 feet 6 inches high, requires 4,100 calories per day.

Sitting increases metabolism 10% more than lying in bed.

The Journal of The American Medical Association, page 825, April, 1918, gives the following tables:

Table 1.—Extra Calories Per Hour Attributable to Occupations of Men.

Occupation of Men	Extra Calories of Metabolism per Hour Due to Occupation
Tailor	44
Bookbinder	81
Shoemaker	90
Metal worker, filing and hammering	141
Painter of furniture	145
Carpenter making a table	164
Stonemason chiseling tombstone	300
Man sawing wood	378

The extra calories per hour attributable to occupation may be given as in Table 1.

Table 2.—Extra Calories Per Hour Attributable to Occupations of Women.

Occupation of Women	Extra Calories of Metabolism per Hour Due to Occupation
Seamstress, needle work	6
Typist, 50 words per minute	24
Seamstress, using sewing machine	57
Bookbinder	63
Housemaid (moderate work*)	81
Laundress (moderate work*)	124
Housemaid (hard work*)	124
Laundress (hard work)	214

*Cleaning windows and floors, scouring knives, forks and poons, scouring copper and iron pots.

Milk is a typical food, giving 12% available food, or one ter gives 1,000 mils or 120 grams nourishing food, or 1 lb. od @ 12c a liter, equals 50c.

Then the following at the relative prices equals in food alue 50c worth of milk: Veal @ 24c equals $1.07, steak @ 5c equals 84c, beef ribs @ 25c equals 91c, chicken @ 35c quals $2.42, pork ribs @ 25c equals 60c, pork chops @ 25c quals 83c, lamb leg @ 22c equals 75c, lamb chops @ 25c quals 62c, halibut @ 25c equals $1.38, eggs @ 44c equals 1.22.

1 liter of milk equals the following in food value: 8 eggs, lbs. salt cod, ¼ lb. canned baked beans, 2¼ lbs. potatoes, ½ lbs. onions, 2-5 lbs. ham, 4⅛ lbs. beets, 5¾ lbs. cabbage, lbs. spinach, 7 lbs. squash, 5½ lbs. turnips, 7 lbs. tomatoes, lb. porterhouse steak, 2 lbs. chicken and 4-5 lbs. pork.

ONE GALLON OF MILK CONTAINS:

Water—3 quarts.

Sodium—5.2 ounces.—To neutralize acids in the blood.

Fat: Soluble—5 ounces.—Energy, heat, growth.

Lime—75 grs.—For bones and teeth (milk contains most me of any food).

Potash—70 grs.—To prevent the body fluids from turn- g acid.

Chlorine—56 grs.—To make acid for the stomach and lts for the body.

Phosphorus—36 grs.—For repair of bones and teeth.

Butter Fat—29 grs.—For body and energy.

Magnesia—7 grs.—For the body fluids.

Milk Sugar—6.8 ounces.—Makes galactose for brain food.

Milk Protein—4.9 ounces.—Best of all the proteins for ood and muscle.

Sulphur—2 grs.—To purify the blood.

Iron—½ gr.—To make red blood.

Malted milk is formed by combining whole milk with ie liquid separated from the mash of ground barley and heat flour, with or without the addition of sodium or potas- um bicarbonate, in such a manner as to secure the full enzy- ic action of the malt extract and by removing the water. he resulting product contains not less than 7½% butter fat, id not more than 3½% moisture.—Food Inspection Divis- n, 1917.

In examining malted milks, temporary mounts are made, using a mounting medium of equal parts of glycerin, alcohol and water. Oil mounts are necessary as the water soluble constituents as sugars et al., dissolve in mounts containing water.

On account of the different manufacturing processes, the preparations vary in composition. Usually the following substances are found: Powdered skim or whole milk; powdered malt, dried malt extract, bread products, glucose, cane sugar, various starches, gelatin, gums and diastases.

Skim milk powder varies in color due to the method of manufacture; if it is prepared in open steam kettles, a golden yellow, to dark orange, color is produced, depending on the amount of heat used. This form cannot readily be reduced to a fine powder. Vacuum pan and spray methods yield a fine white powder, more readily soluble; hence this method is preferred. The milk powder appears as spherical irregular masses, composed of aggregations of fat globules held together as an albuminous mass; some of the masses are without fat globules and are granular.

Lactose will crystalize out in about 2 hours in glycerin and water mounts, and may fill the whole slide with crystals This in an indication of skim milk in the preparation.

Whole milk powders prepared by the open kettle metho are deep in color and cannot be reduced to a fine powder Spray-whole milk powders are a cream color and also canno be reduced to a fine powder. These are not as soluble ir water as skim milk powders and are more expensive. Th spray or vacuum processes produce smaller particles, an are more regular in their spherical outline. Fat globules ar seen in the masses and are of a pearly color and granular Lactose will not readily crystallize. Crystalization may no take place for 24 to 48 hours. The fat globules in the ope kettle method, are very large and almost cover the surfac of the masses, when full or whole milk is used; but if skim milk is used, the fat globules are fewer and smaller.

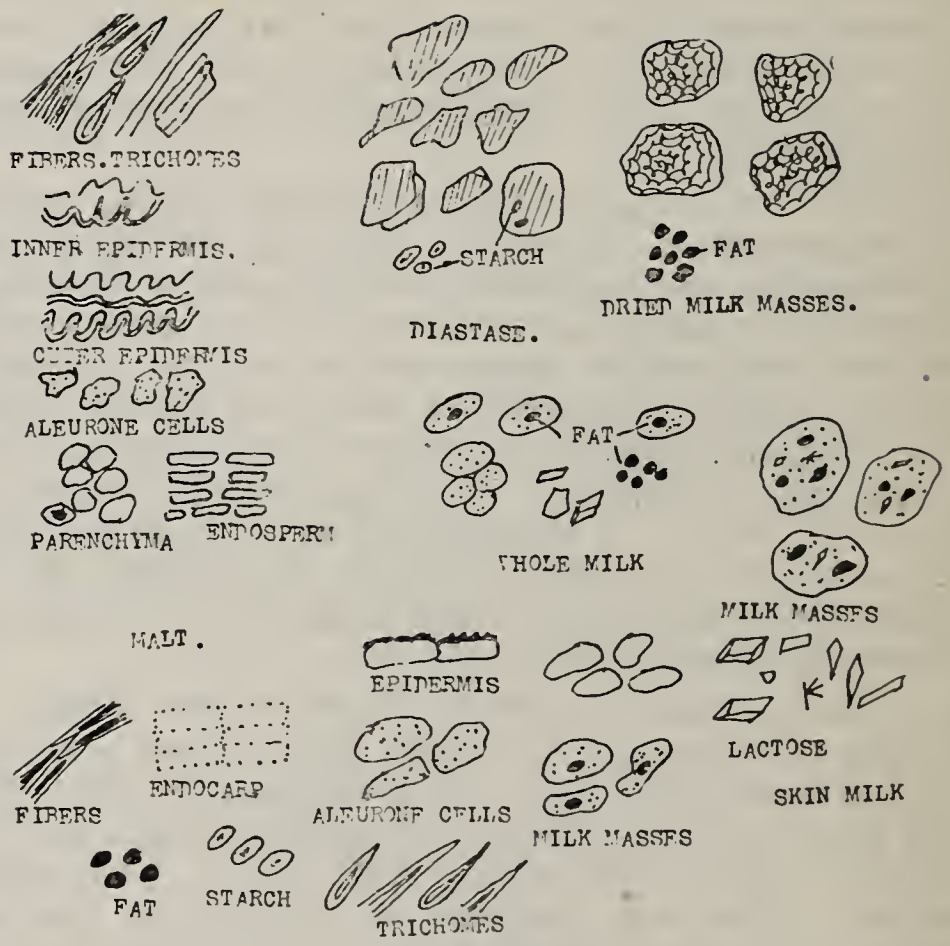
In malt powders, the cells of barley are present; th parts of which in the order of their relative amounts are Starch, (which may be changed by the amylolytic ferments and appear as partly dissolved, the hilum being the first par digested; and in an aqueous iodine solution gives the amylo dextrine and maltodextrine reaction of purplish red), the tri chomes are of two kinds—short thick walled, or long thi walled, usually broken. The outer epidermis has thick wav walls common to the Graminaceae; and stomata; short thic

alled warty trichomes and trichome scars. The inner epi-ermis has whitish cells, thin walled and angular, short thick alled trichomes, and nearly square stomata. The pericarp lls are two rows in thickness, one heavy and one light row, in walled, and rectangular. The endosperm parenchyma is pical parenchyma. The epicarp cells are whitish, long, ngular and thin walled, with trichomes but no stomata. leurone grains are thick walled, angular and full of gran-ar nitrogenous matter.

Powdered malt extract is often used and sold as malted ilk, but on account of its readily taking up water, it must kept absolutely dry or caking occurs, and a secondary rmentation may take place which renders it unfit for use nd produces disagreeable odors and taste. It is readily luble in water and is composed of masses of diatase, which pear as irregular, angular light yellow colored fragments -some may show striations. As it is soluble in water, oil ounts must be made. It contains starch in small amounts. hey all should show diastase action on the grains. A few sette crystals appear, whole and fragments of aleurone rains, parenchyma cells and trichomes.

Mixed malted milks are those which in the process of aking, have undergone fermentative changes and are com-sed of powdered milk and malt preparations; hence the arch to a great extent is digested. The fat globules are sin-e and small, and are free in the preparations. The masses e irregular. The malt particles are yellowish; the cell rticles few in number; the milk masses are irregular; some ntaining fat globules; parenchyma cells are small and oken. Aleurone grains remain practically unchanged in ructure. Trichomes are broken, seldom whole; diastase ap-ears in irregular light yellow, striated angular fragments -water soluble. Starch grains are few and appear as partly gested.

In the imitation malted milk, baked products such as ead crumbs and corn starch, gelatin, gums, et al., along ith glucose, may make the preparation thicker in consist-ncy, and are readily distinguished under the microscope.



The food products turned out by factories in the United States in 1917 are valued at $2,300,000,000. The capital invested in the food industry is $1,000,000,000. The cereals for 1917 were estimated at $8,000,000,000. The United States is the greatest wheat growing country in the world. Russia is second. England, France and Italy do not grow enough wheat

for their needs. All the cereals, except buckwheat, such as wheat, corn, rye and rice, belong to the grass family. These grains are grown in all parts of the world. The human food part is the seed. Herbiverous animals consume all the grasses in making food for us. The sources of food for mankind and lower animals are closely related, we eat our grass by proxy. The animals manufacture food for us.

The food nutritives are the proteins, carbohydrates, organic fats, vitamins, food hormones, and inorganic or mineral matter.

The proteins are egg albumen, red and white meats, casein, gelatin, gluten, legumin, fish meat, peptones, etc.

The carbohydrates are glucose, dextrose, grape sugar, fructose sucrose, lactose, maltose, starch and those of the grains and vegetables.

Organic fats are butter, lard, beef fat, fowl fat, mutton fat, fish oil, oleomargarine, olive oil, cotton seed oil, peanut oil, corn oil, sesame oil, almond oil, cacao butter, cocoanut oil, nut oils, etc.

Vitamins and food hormones. The water soluble kind is found in cereals, fruits, vegetables, meats and milk. The fat soluble kind is found in butter, yolk of egg, roots, leaves, milk and codliver oil.

Inorganic or mineral matter is found in water and in foods and includes calcium compounds, magnesium, iron, sulphur, phosphorus, sodium, potassium chlorine, etc.

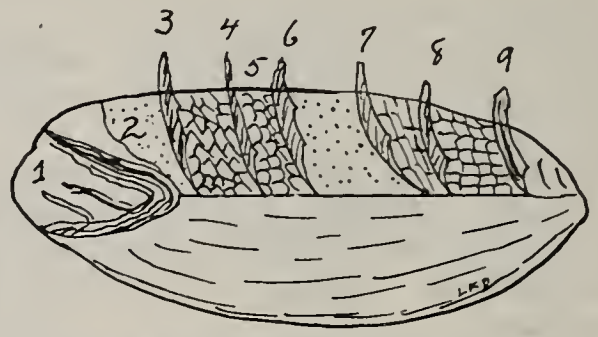
There is more protein in wheat than in corn. Oats and corn contain more fat than rice or wheat; but the food values in calories, measure weight for weight about the same.

Wheat, rye and oats contain about the same amount of proteins. Rice contains more carbohydrates than the other grains. Barley contains the most mineral matter and polished rice contains the lowest mineral matter. On account of the high contents of the carbohydrate in the cereals, they should be combined with other foods, richer in fats and proteins. This we do in various ways. We spread our bread with butter, etc. Sandwiches are made richer in protein and fat by the butter and meat we put in them. Other forms of cooking balance the nutritives.

The deficiency of certain food principles in the system has a way of making their wants known. As in the case of salt and water, animals will travel miles to get them.

Wheat is by far the most important cereal used by man.

Varieties of wheat are red, white, hard, soft, starch, winter and spring. Normally we use annually about 5 bushels per capita. Bread is the basis of the food supply of the world; and on examining the grain of wheat under the microscope the structure appears as



1 TO 5. ENDOSPERM-85 % OF THE BULK. 1. COTYLEDON-1 1/2 % OF THETHE BULK. 2. FLOUR CELLS WITH GLUTEN,STARCH,ETC. 3. ALEURONE GRAINS,MINERALS,VITAMINS,ETC. 4. INNER BRAN COAT-WITH FIBERS AND MINERALS. 5. TO 9. OUTER BRAN LAYERS OR THE COLORING LAYERS-13 1/2 % OF THE BULK.

PARTS OF A WHEAT GRAIN.

Draw a section of an official seed, name the seed and cells. Do this before the next period.

ARTIFICIAL FOODS

All artificial foods should be uniformly and finely powered; they should be free from all micro-organisms (molds, bacteria and yeasts); they should contain the proper constituents in their proper amounts; there should be little or no unconverted starch and the fat should be in small uniform globules.

EXERCISE

Examine the various preparations and report, giving tests and drawings: 1. Starch; 2. Dextrin; 3. Fat; 4. Proteins; 5. Salt crystals; 6. Sugar crystals; 7. Other constituents and cells; 8. Do you consider the specimen under examination a good food? 9. State reason for your answer.

NON-CELLULAR DRUGS

Gummy Exudations.

Round colorless opaque tears or brittle fragments, ACACIA.

Flakes or ribbon bands, yellow white, translucent, horny, TRAGACANTHA.

Balsams.

Brown or reddish, liquid, smoky odor, acrid taste, BALSAMUM PERUVIANUM.

Light brown, semi-solid, not sticky. Odor and taste, BALSAMUM TOLUTANUM.

Gray, opaque semi-fluid, balsamic odor, acrid taste, STYRAX.

Balsamic Resins.

BENZOINUM.

Yellow brown fragments with milk white tears,

Pale brown, separate tears, internally milk white, Sumatra.

Pale brown, agglutinated, internally white, Siam.

Chocolate brown, no distinct tears, Penang.

Oleoresins.

Yellow plastic masses. Odor and taste, TEREBINTHINA.

Clear yellow viscid terebinthinate, liquid, may be turbid, Terebinthina, Larcia.

Clear yellow liquid, on exposure to air solid,
Terebinthina, Canadensis, N. O.

Yellow resinous liquid. Odor and taste, COPAIBA.

Gum Resins.

Friable red brown tears or masses, balsamic odor, acrid taste, MYRRHA.

Irregular pink yellow masses, firm, tears pinkish. Odor, ASAFOETIDA.

Yellow cylinders or tubes, CAMBOGIA.

Green black angular masses, porous, cheese like odor, RESINA SCAMMONII.

Resins.

Angular transparent yellowish masses, RESINA.

Globular pale yellow transparent tears, plastic, Mastiche.

Greenish masses, tough when chewed, glossy fracture, GUAIACUM.

Milk Juices.

Brown lumps, heavy narcotic odor, nauseous bitter taste, OPIUM.

Gray masses, streaked with brown, flexible, taste, Gutta Percha.

Gray brown cakes or fragments, fracture waxy, narcotic odor, bitter taste, LACTUCARIUM.

Prepared Artificially.

Irregular brown lumps, saffron odor, intense bitter taste, ALOE.

Yellowish microcrystalline powder or acicular crystals, bitter, ALOINUM.

Cubical or rectangular gray brown pieces, bitter, astringent, GAMBIR.

Small ruby red angular fragments, adheres to teeth when chewed, KINO.

Dark brown masses, brittle, leaf fragments, Catechu, N. O.

Brown yellow micro-crystalline powder, tasteless and odorless, CHRYSAROBINUM.

White crystalline translucent, tough mass, odor, CAMPHORA.

Saccharine Substances.
3 edged yellow white porous pieces, crystalline, taste sweet then acid, MANNA.
Yellowish, syrupy, sweet taste, GLUCOSUM.
Coloring Substances.
Vegetable.
Lichens.
Purple red powder, Persio.
Rectilinear bluish cakes, granular or powder, violet odor, Litmus, N. O.
Brittle blue purple masses, copper color when pressed with fingernail, Indigo, N. O.
Animal.
Gray white angular granules, COCCUS.
Mucilaginous.
Wrinkled, folded, flat pieces, AGAR.
Burned wood,
Black pieces, showing wood structure, non-gritty, CARBO LIGNI.
Solid or semi solid fats or oils,
Yellowish white, chocolate taste, OLEUM THEOBROMATIS.
Mineral.
White, waxy, no granular structure, PARAFFINUM.
Yellowish, greenish, fluorescence, ointment consistency, PETROLATUM.
Animal drugs.
Flies or beetles.
No wings, gray white angular granules, COCCUS.
With wings, green or copper luster, CANTHARIS.
Parts of animals.
Solid animal substances.
Yellow waxy cakes or lumps, CERA FLAVA.
White thin waxy cakes, CERA ALBA.
White semi-transparent crystalline, unctuous, CETACEUM.
Hard white glossy masses, ACIDUM STEARICUM.
White soft fatty masses, SEVUM PRAEPARATUM.
Black gritty powder, Carbo Animalis, N. O.
Cylindrical crystalline masses, SACCHARUM LACTIS.

Opaque rectangular flexible sheets, GELATINUM.
Black irregular glistening grains, odor, MOSCHUS.
Cream colored amorphous powder, taste, PANCREATINUM.
Gray yellow powder or scales, saline taste, aromatic odor, RENNINUM.

Semi-solid.

Soft white unctuous fatty substance, ADEPS.
Yellow white tenaceous ointment like. ADEPS LANAE.
Light yellow amorphous, powder, odor, SUPRARENALUM SICCUM.
Light yellow amorphous powder, odor, THYROIDUM SICCUM.
Gray amorphous powder, odor, HYPOPHYSIS SICCA.

Liquid.

Syrupy, sweet aromatic liquid, MEL.
Viscid green brown, intensely bitter, FEL BOVIS.
Pale yellow, oily, peculiar odor, Oleum Adipis, N. O.
Pale yellow, oily, peculiar odor, Oleum Adeps, N. O.

SOME OF THE NON-CELLULAR DRUGS

GUMS.

Acacia.—This contains little or no starch; but in some the starch may be altered; few tissue fragments; soluble in water; precipitated by lead acetate or ferric chloride solutions—not blue with iodine. With water it forms a gummy adhesive mass.

Tragacantha.—Swells in water, and is not dissolved, contains few starch grains, single or two to four compound. When warmed with patassium hydroxide solutions, it turns yellow; and then when iodine T. S. is added a blue precipitate results. In liquid state when alcohol is added and then iodine T. S. no blue color appears.

Indian gum in glycerin mounts shows mold, spores, wood fibers, calcium oxalate, and large brownish yellow masses.

BALSAMS.

Styrax.—Is insoluble in water, but soluble in 60% warm alcohol; when boiled with potassium dichromate solution, then adding sulphuric acid, the odor of bitter almonds is developed. See your U. S. P. for other tests.

BALSAMIC RESINS.

Benzoinum.—Appears in temporary mounts colorless, or wine colored fragments, with a few rosette crystals, few or

no cells; if any, they are of the phloem. Sublimation in the dished slide yields crystals of benzoic acid on the cover glass.

Sumatra Benzoin.—Yields by sublimation crystals in plates or small rods. Boiled with acidulated potassium permanganate solutions, no odor of benzaldehyde develops.

Siam Benzoin.—Treated as above gives benzaldehyde odor, due to the cinnamic acid the drug contains, On sublimation the crystals are long rods; and do not polarize light as does the Sumatra sublimate.

OLEORESINS.

Terebinthina contains cells of the phloem. When mixed with water and placed in the sunlight for 15 hours, crystals of pinol hydrate develop. These are soluble in alcohol, giving a straw color liquid. And when it is warmed in water, the fragments melt and form masses.

Terebinthina Canadensis.—Contains few or no phloem cells. When it is mixed with equal parts of sulphuric acid a red color develops.

GUM RESINS.

Myrrha.—Forms a bright yellow emulsion when rubbed in water; if nitric acid is now added a purple color results. If 15 parts of glacial acetic acid is mixed with one part of Myrrha a purple color results; then when sulphuric acid is added a pale rose color results. In glycerin mounts or fixed oil mounts, the masses show brownish yellow globules of oil, a few tissues (bast and stone) and a few starch grains.

Asafoetida when triturated with water yields a milky emulsion; then if an alkali is added a yellow solution results. Alcoholic solutions to which is added hydrochloric acid, gives a blue color, changing to green on standing. Glycerin mounts show brownish masses streaked with grey. They are opaque and milky on the edges from the oil. Some resin is present; test with cupric acetate T. S.

Cambogia in glycerin mounts consists of angular or irregular bright yellow masses containing little or no starch, a few tissue fragments, and forms a yellow color with water; a red solution with alkalies. It is slowly soluble in chloral T. S.

Resina Scammonii.—Contains root tissues, a little or no starch. When rubbed in water, it forms a milky greenish emulsion—no effervescence with hydrochloric acid, and no blue color with ferric chloride T. S. (Absence of Guaiacum). No red color is formed when sulphuric acid is added (absence

of rosin). Crystals of scammonic acid are formed when alkalies are added.—Iodine T. S. forms with the drug a syrupy mass, with rounded fragments (false will not do this).

RESINS.

Guaiacum when heated on a dished slide gives an odor of benzoin. It is soluble in alcohol with a brown color; then when ferric chloride T. S. is added a blue color changing to emerald green and finally to yellow is produced. A few xylem cells may be present. But no green color with cupric acetate T. S. (absence of rosin).

Mastiche is soluble in ether, chloroform and nearly so in alcohol, and this solution is acid to litmus.

MILKY JUICES.

Opium. When sulphuric acid is added to Opium, crystals of morphine sulphate separate out; then if potassium dichromate T. S. is added a blue solution results, and after standing it turns green. Opium has leaf or poppy fragments (capsule) consisting of thick walled epidermal cells, etc. See page on Opium.

Smyrna opium contains many capsule epidermal cells.

Indian opium contains few or none.

Persian opium contains few or none, but contains much starch.

Gutta Percha.

Lactucarium when rubbed with water forms a milky solution—partly soluble in alcohol or ether. It contains no starch but crystals of lactusin. It is grey brown with alkalies, turning red to brown, then dirty brown. It has no reaction with sulphuric acid.

PREPARED ARTIFICIALLY.

Aloe.—Contains leaf cells, the fragments being yellow to brown; and when sulphuric acid is added a deep red solution results (Aloin test); then if a drop of potassium dichromate T. S. is added an olive green color turning to blue results. Alcoholic solutions with ferric chloride T. S. give a brown-green color (Aloin test). Nitric acid gives a yellow or red brown color. Aqueous solutions with a solution of sodium bromide give a fluorescence; and characteristic acicular and prismatic crystals separate out with alkalies and the fragments are reddish.

Aloinum.—With alkalies forms a yellowish solution, changing to red with a green fluorescence. Dilute aqueous

olutions of Aloin (except Natal) with borax, give a green olution with a green fluorescence; or with silver nitrate T. . and then ferric chloride T. S. a dark brown green color esults (Cape aloin gives a green).

Dry aloin with nitric acid gives a carmine red. With alalies a red solution is given, turning to green with a red uorescence. With sulphuric acid a yellow red solution reults; then when potassium dichromate T. S. is added an olive reen color changing to blue. Alcoholic solutions with ferric hloride T. S. give a brown green.

Socrotine aloe with nitric acid gives a yellow to red rown. With hot water solutions and alkalies or acids a red precipitate is formed. In glycerin mounts, typical acicular rystals and angular fragments are seen.

Curacao aloe with nitric acid gives a deep red, as with socrotine and also with alkalies. In expressed oil of almonds t appears as black brown fragments, some being opaque.

Zanzibar aloe with nitric acid gives a dark yellow color.

Barbadoe aloe with nitric acid gives a cherry red color, which soon fades; if it is then boiled with oxalic or picric acid T. S. crystals will form. In glycerin mounts many acicular crystals form. A pink color is produced in dilutions of 1 in 1,000, when a drop of fresh potassium ferricyanide solution is added.

Cape aloe contains no aloin, hence no permanent red color with nitric acid; but a play of colors may be seen, at first red brown, then purple, and finally greenish. Nitric acid intensifies the color of the drug. It is reddish with alkalies. In glycerin large rectangular crystals form. In oil of almond the masses are angular and of a bright yellow color.

Natal aloe with nitric acid gives a permanent crimson color. It gives a deep blue color with sulphuric acid. It forms in water, glycerin or alcohol mounts, rectangular crystals.

Uganda aloe or crown aloe gives yellow to brown color with nitric acid.

Gambir in chloral T. S. forms acicular crystals, rapidly dissolving and forming prismatic plates. Cells of the phloem of twigs and leaves may be found. In chloral T. S. needle shaped crystals form. Aqueous solutions with ferric chloride T. S. turn green.

Catechu in water mounts appear as dark brown blackish fragments, and acicular crystals are formed. It is precipi-

tated with copper sulphate solution or silver nitrate T. S. It is green with ferric chloride T. S. Cell of the xylem may be found.

Kino when mounted appears as red glass-like fragments, with a few phloem cells. It turns blackish green with ferric chloride T. S., or violet with ferrous salts. Prismatic crystals separate out when standing in water mounts.

Santoninum with alcohol turns a deep red and dissolves. With sulphuric acid, then with ferric chloride T. S. and warmed, a red color, then violet appears. When alcoholic solutions of the drug are warmed and potassium hydroxide solution is added a violet red color results.

Elaterinum in mounts appear as gray brown fragments. When heated with a solution of phenol and cooled, then sulphuric acid added, a deep red color is produced.

Goa powder contains a few xylem cells. In chloral T. S. the fragments are wine color, crystals or prisms are formed. Also with alkalies or sulphuric acid, crystals are formed and the fragments are deep red with green fluorescence. It is precipitated with an excess of water.

Chrysarobinum appears in mounts as needle, prisms and often rosette or aggregates. In dilutions of 1 to 20,000 a brown color is obtained. It is not precipitated by ferric chloride T. S. When sulphuric acid is added to the drug a red color results.

RESIDUE.

Resina in alcohol mounts gives a straw colored liquid and crystals of abietic are formed; if water is added a milky solution is produced; and if it is now heated the rosin will form in masses. It is soluble in hot alcohol.

Halpin's Resin Test.

Two reagents are used. 1. Phenol 1 part, carbon tetrachloride 2 parts. 2. 1 part of bromine and 4 parts of carbon tetrachloride. The test is made by placing a small amount of the resin on a porcelain dished slide in 1 mil of the No. 1 solution. Place 1 mil of solution No. 2 near that of No. 1, but not touching, close enough so that the vapors will flow across solution No. 1 containing the resin.

Colophony gives a green, then blue, then violet, on standing purple, finally indigo-blue.

Damar gives a brown, then lilac, then brown, finally gradually to red brown.

Elemi gives indigo, then blue, which deepens and finally becomes purplish.

Kauri gives an azure blue, then rapidly a purple, finally a dark olive green.

Manilla (alcohol soluble) gives a brown green, then violet, then purple, finally a chocolate brown.

Mastic gives a red brown, the parts nearest the reagent a carmine.

Sandarac gives a permanent lilac, and violet at the farthest point from the reagent, turning to violet brown.

Shellac gives no reaction.

Zanzibar copal gives a light brown, then brown violet, then a chocolate brown, finally a violet.

PASTE.

Guarana contains characteristic starch, some of which may be altered, and seed cells. If hydrochloric acid is added to a small amount of guarana, then gold chloride T. S. is added and the slide set aside for at least 3 minutes, crystals of caffein in the shape of plates, needled and branches will form along the edges.

SACCHARINE EXUDATION.

Manna contains cells of the phloem and prisms of fraxin. Manna is nearly all soluble in water or alcohol. If a little manna is heated in alcohol on the dish slide and cooled, mannite crystals will form.

EXCRESCENCE.

Galla, in powdered form, when in glycerin mounts, shows stone cells, crystals of gallic acid, some starch, laticiferous cells and thick walled parenchyma. See description under Yellow Powders.

Talcum Purification in water mounts shows the characteristic hydrous magnesium silicate forms.

Terra Silicea Purificata in water mounts contains the frustules and fragments of diatoms.

KETONE.

Camphora.—Place 0.1 gram natural camphor on a dish slide and add 10 drops of a solution of vanillin, and then a few drops of hydrochloric and sulphuric acids, cover with a cover glass, set aside for one half hour, then examine, the solution will be pink, turning in 2 hours to green and finally to blue. The synthetic camphor will give a permanent yellow color—camphor crystalizes from alcoholic solutions with the characteristic crystals.

RUGS WHEN FRESHLY POWDERED WHICH ARE OF A GREENISH COLOR

This group of drugs is composed chiefly of Leaves ıd Herbs—a few Barks and Seeds. The drugs are of a eenish color when freshly powdered. The fineness of the wder, the age of the powder and the surface on which the wder is examined, influence the color.

This group is divided into two parts: 1st. Those which ntain crystals, and 2nd. Those which contain no crystals.

Drugs containing crystals of calcium oxalate.

A. These contain glandular and non-glandular trichomes, calcium oxalate in rosettes.

Cannabis, with the cystolith non-glandular, retort shaped trichome containing calcium carbonate in its base, (effervescing with HCl), oil, tannin, tracheae, laticiferous with brown contents, two kinds of glandular trichomes. Greenish brown sphenoidal micro-crystal cells. Stone cells non-lignified, palisade like in shape, filled with air are from seed.

Eriodictyon has non-glandular "cork screw" shaped trichomes on the lower surface. It contains large epidermal cells, many glandular 1 to 8 celled head hairs, narrow palisade in rows of 2, a few mesophyll cells. The cork cells from the stem are oval and the stem contains 10 to 20 rows of thick bast, sieve, tracheae and wood cells. The medullary rays are one cell wide. The pith is large and lignified.

Stramonium is characterized by the diagnostic trichomes (see page on). The stomata are elliptical with 3 neighboring cells, the mesophyll contains small chloroplasts, crystals in rosette, rod or sphenoidal micro-crystals. The stem contains tracheae, wood parenchyma, long narrow collenchyma with irregular walls and no bast.

Humulus is diagnosed by the non glandular unicellular bent, thick walled, trichomes and the glandular 3 celled stalk and colorless multicellular head or the short 4 celled stalk and multicellular yelow glandular head.

Pyrethrum Flores—Insect powder, contains the "T" shaped trichomes, 2 celled stalk and needle like apex and pollen. Fragments in chloral T. S. turn a rose color—with sulphuric or hydrochloric acid they turn a yellowish green. With nitric acid a yellowish

brown; and with ferric chloride, a greenish black. The corolla cuticle is striated, the inner cuticle is papillated (see pages on unclassified drugs). Sessile glandular 2 to 8 celled secretion headed trichomes.

a. No glandular trichomes, but with simple trichomes.

Pilocarpus contains tannin and oil in secretion cells, the epidermal cells are 5 to 6 sided, with characteristic non glandular trichomes, which are thick walled, curved or bent, with centrifugal projections. It contains a few wood cells. The bast are thick walled and slightly lignified. The trichomes and shape of the stomata are diagnostic of the kind of Pilocarpus. (See pages on leaves).

Tea is diagnosed by the unicellular long thick walled "cane shaped" trichomes, the colorless stone cells, ("idioblast") trichome scars and stomata with 3 to 4 neighboring cells.

b. No trichomes.

Buchu has hesperidin (a better principle) crystals and mucilage in the epidermal cells, and many oil cells.

Eucalyptus has many palisade which replace nearly all other cells in the mesophyll, yellow brown schizogenous resin or oil cells, cutinized epidermis, stomata, some may be sunken or filled with resin, and a few lignified bast.

Chimaphila contains parenchyma with red brown masses of tannin. The lower epidermis has thick walled cells with broadly elliptical stomata. And cells with a purple brown pigment, which turns yellow with acids and bright green with alkalies.

Conium has characteristic aleurone grains, yellow chromoplast cells, yellow isodiametric pericarp cells, long thin walled bast, and gives a mouse odor on moistening and rubbing in the hands.

Granatum contains rosette and monoclinic crystals, in crystal fibers and cells—starch and tannin in parenchyma cells, stone cells and whitish lignified cork.

B. Drugs containing monoclinic prisms only, and trichomes.

Hyoscyamus has the diagnostic trichomes, (see pages on leaves), also the four different shaped crystals.

a. No glandular trichomes.

Senna has a diagnostic 1 celled curved trichome, with slight centrifugal projections and slightly cutinized. Fragments are reddish with alkalies.

Castanea contains the characteristic trichomes, and

much tannin. (See pages on trichomes).

Uva Ursi has a few crystal fibers, a few curved 1 celled trichomes, spongy parenchyma with yellow brown tannin masses and characteristic bast. Cellls containing a carbohydrate and chlorophyll, give a blue black color with ferric chloride T. S. The epidermal cells are polygonal. The lower surface has broadly elliptical stomata with 5 to 8 neighboring cells.

Cardamomum contains many brown stone cells from the seed coat, epidermal cells with oil and suberized walls. Compound starch. Elliptical stomata with 3 to 4 neighboring cells. Unicellular 2 to 10 celled trichomes. It may contain a few glandular 1 to 4 celled stalk and 6 to 10 celled head trichomes.

Cardamomi Semen has endosperm and perisperm cells, oil and compound starch. In chloral T. S. crystals separate from the powder.

Hamamelidis Folia contains trichomes in groups of about 15, spreading from one base, thick walled, unicellular and with a brown content. The parenchyma contains tannin and oil. The mesophyll has crystals and a few crystal bast. The lower surface has stomata, which are narrow, elliptical and with 2 to 4 neighboring cells.

b. No trichomes.

Coca has a papillated lower epidermis, elliptical stomata, annular tracheae, a few palisade and parenchyma with monoclinic crystals. See pages on leaves.

Guaiacum contains resin masses, which in chloral T. S. are wine colored—cells of the xylem, large tracheae with resin, irregular wood parenchyma, some with monoclinic crystals or yellowish red resin, and medullary rays 4 cells wide.

c. Sphenoidal micro-crystal cells (cryptocrystalline cells).

Belladonnae Folia has the diagnostic hairs, green crystal cells, few xylem elements, a few small starch, sometimes pollen and no raphide crystals.

Hyoscyamus. When no description is given, it signifies that description has been previously given.

Cannabis. See note under Hyoscyamus.

Tabacum has the crab claw shaped trichomes, brown crystal cells, mesophyll with a brown green content, large stomata with 2 to 3 neighboring cells and striated epidermis.

Stramonium. See note under Hyoscyamus.

Solanum carolinense or horse nettle, characteristic trichomes (see pages on trichomes).

Dulcamara has long unicellular trichomes, non-lignified bast, tracheae with bordered pores, and all cells of a typical stem.

2. Drugs with no calcium oxalate.

A. Those with trichomes.

Digitalis is diagnosed by the collapsed trichomes. It contains no crystals or starch.

Eupatorium has ellipsoidal pollen with many centrifugal projections. The tracheae are thin walled and the bast are non-lignified. Diagnostic trichomes which are non-glandular 2 to 8 celled thin walled, finely striated, the end cells are acute or rounded; and the glandular 2 to 8 cells in a double row with a 2 celled head, or a short stalk with a 4 to 12 celled glandular head.

Grindelia contains tracheids, spherical pollen with centrifugal projections, 3 pored, elliptical stomata projecting above the epidermis, multicellular trichomes, mesophyll cells with chlorophyll, large colorless walled storage cells with brown resinous masses, which are precipitated in glycerin mounts, non-glandular trichomes, some of which may contain narrow rod shaped crystals.

1. Those with diagnostic glandular trichomes.

a. 1 to 2 celled head.

Mentha Piperita has smooth spherical pollen, vessels, diagnostic next to the end cell, collapsed trichome, non-glandular trichomes, 1 to 8 celled and papillose, some with menthol crystals—glandular trichomes, 1 to 3 celled stalk and 1 to 8 celled head, bast long thin non-lignified.

Mentha Viridis with the epidermal cells containing purple chromoplasts, the corolla and style contain sphere shaped carbohydrate crystals, the pollen is smooth and oval, non-glandular trichomes, 1 to 6 cells, and glandular have a 1 celled stalk and a 1 celled head.

Hedeoma has a 2 to 3 celled thin walled non-glandular trichome, curved, with many centrifugal projections, glandular trichomes with 1 celled stalk and 2 to 8 celled head. Spherical pollen. The ves-

sels and bast are long, thin walled and lignified. Irregular masses of crystalline carbohydrates are seen in the epidermal cells.

Salvia has characteristic 1 to 6 celled non glandular trichomes; the basal cell is trick walled and contains air—glandular trichomes with 1 to 4 celled stalk and 1 to 8 celled head. The 8 celled trichomes are usually brownish. The upper epidermal cells have thick walls, while those of the lower epidermis are thin walled and wavy.

Scutellaria has the 1 to 3 celled non-glandular trichomes with centrifugal projections and tapering from a sharp apex to a blunt base and slightly curved. Fragments of the corolla are pink with chloral T. S. The elliptical stomata have small openings. Glandular trichomes have a 1 to 2 celled stalk and 6 to 8 celled head.

Marrubium has twisted 1 to 15 celled non-glandular trichomes in groups of 5 to 16. The glandular trichomes have a 1 celled stalk and 2 to 9 celled head. The tabular epidermal cells are thin walled and wavy. The bast are thin walled and non-lignified.

2. Those having no glandular trichomes.

a. With Pollen.

Tanacetum has few trichomes. They are 4 to 5 celled and with a yellow brown content. Glandular trichomes are from the achenes. The pollen is spinose and spherical. The bast is thick walled and not pored.

Lobelia has smooth ellipsoidal pollen, branched laticiferous with granular contents and one celled conical elongated trichomes. Some of the epidermal cells are raised gland-like. The stomata are elliptical.

b. No Pollen.

Matico is characterized by the 1 to 8 celled trichomes, with a pointed apical cell—rounded yellow resin masses and oil glands.

Scoparius has vessels, bast—thick walled 1 cell trichomes, and elements of the phloem and xylem. It may contain a few leaf cells.

Chelidonium gives a yellow color to water. It has small reniform seeds and latex tubes. The pollen is smooth and spherical and 3 pored, no starch or

crystals. The trichomes are 6 to 8 celled, swollen at the dividing walls. The stomata are oval. Cells of the leaf are found.

3. Those having no trichomes.

Staphisagria has elongated thick walled bast, which in the transverse view are ovate. The parenchyma has fixed oil, aleurone and pigments, and the cells are thick walled with reticulate pores.

Sabina is characterized by the hypoderm cells, long fibers, narrow tracheids, starch and oleoresin.

DRUGS WHEN FRESHLY POWDERED WHICH ARE OF A YELLOWISH COLOR

This group of drugs includes Roots, Rhizomes, Barks, Woods, Seeds, Fruits and a few Flowers. It is divided into two parts, 1st. Those drugs which contain tissue or cells; 2nd. Those which contain few or no tissues or plant cells.

1. Drugs containing plant tissue or cells.
   A. Those containing starch.
      a. Containing calcium oxalate.
         1. In rosettes.

Frangula has crystal thick walled bast with monoclinic prisms, spherical starch, parenchyma with yellowish content which turns reddish with alkalies and many rosette crystals.

Galla—Aleppo contains single or compound starch, isodiametric thick walled stone cells with tannin, parenchyma with tannin, laticiferous and dark reddish crystals of gallic acid.

Galla—Chinese contains few crystals, large starch, non-glandular trichomes, laticiferous and many stone cells. In glycerin, acicular crystals of gallic acid separate out—these crystals are yellow to red or gray green.

Jalapa has ellipsoidal or oval starch excentric, 1 to 3 compound, some of which may be altered, the laticiferous and parenchyma have yellowish walls, brownish resin cells and tracheae.

Pulvis Jalapae Compositus appears as angular fragments, some are soluble in water or chloral T. S.—fragments of potassium bitartate crystals and cells of Jalapa.

Rheum consists of about 1-3 its weight of rosette crystals of calcium oxalate. It contains starch, 1 to 4 compound. The powder is reddish with alkalies. If the drug has been exhausted it shows altered starch. The medullary ray cells are dark brown, the parenchyma are light brown.

Pulvis Rhei Compositus contains cells of Rheum and Zingiber, fine particles of magnesium oxide. It effervesces with chloral T. S. and many fragments are deep red with alkalies.

Rhapontic Rheum has the glucoside rhaponticin, which with sulphuric acid turns purple to orange. In alcoholic solutions crystals separate out when ether is added.

b. Calcium oxalate in monoclinic prisms.

Curcuma has altered starch in masses, yellow oil or resin secretion cells—the contents are insoluble in alcohol or chloral T. S. When alcohol is added to the powder, then adding a borax solution, a cherry color is produced; if ammonia is now added the color will change to blue black. A few tracheae may be found. Many of the parenchyma cells have suberized walls.

Calumba is characterized by having stone cells containing one or more prisms. The starch is usually single, irregular, oval and excentric and a few 2 to 3 compound grains may be seen, sphenoidal micro-crystal cells and cells of the phloem and xylem. The cork cells are small and large.

Frangula. See previous description.

Gelsemium has spherical starch, long narrow lignified bast; tracheids and the epidermal and parenchyma cells contain an alkaloid which is precipitated with iodine T. S. and potassium iodide T. S. as a brown red precipitate. With nitric acid these cells are dark yellow and the alkaloid appears as needle shaped crystals. A yellow precipitate is formed with picric acid.

Quassia contains large tracheids, long wood fibers, 4 to 6 sided crystals, (many of which are found in the medullary rays), sphenoidal micro-crystal cells, a few starch, which are elliptical in shape. No stone or cork; these are from the bark and show adulteration.

Jamaica quassia has tracheae in groups of 2 to 5, medullary rays 1 to 5 cells wide and 10 to 20 long, little starch and many crystals.

Surinam quassia has tracheae in groups of 3 to 4, medullary rays 1 to 4 cells wide and 10 to 30 long, much starch and few or no crystals.

3. Contain crystal fibers.

Jamaica quassia.

Glycyrrhiza—Spanish is characterized by the yellow crystal fibers, both bast and wood, monoclinic crystals, red brown cork cells and cells of the phloem and xylem.

Glycyrrhiza-Russian contains no cork, but cells of the xylem only.

Pulvis Glycyrrhiza Compositus, when mounted in

chloral T. S. shows the characteristic cells of glycyrrhiza and senna, sulphur granules and crystals of sugar. Aqueous mounts to which alkalies have been added, show fragments colored yellow red, changing to red brown. Senna has the curved unicellular hairs, elliptical stomata, with 2 neighboring cells and crystal fibers.

4. Contain raphides of calcium oxalate.

Ipecacunha-Rio contains many intermediary fibers, variously marked tracheids and many characteristic ellipsoidal 1 to 6 compounds starch. A few stone cells with branching pores. In alcohol and water mounts, when picric acid is added a yellow precipitate forms; then if hydrochloric acid and potassium chlorate T. S. are added an orange red color with a blue fluorescence results. A precipitate is formed with potassium mercuric iodide T. S.

Cartagena ipecac contains very large starch cells.

Phytolacca has long bast, many tracheids, starch, sphenoidal micro-crystal cells, and cells of the phloem and xylem.

Veratrum contains vessels, long thin walled bast, elliptical 1 to 3 compound centric starch, some of which may be altered, endoderm cells, fundamental tissue and cells of the xylem.

Veratrum Viride with concentrated sulphuric acid gives a yellow red color.

Veratrum alba with concentrated sulphuric acid gives a dull red color.

c. Containing no calcium oxalate or stone cells.

Zingiber has diagnostic bast and starch—secretion cells with light yellow suberized walls and containing yellowish oil or resin, which with sulphuric acid turns a golden yellow in fresh ginger and a deep brown black in old ginger—cells of the xylem, fundamental tissue and endoderm are present.

African ginger contains much cork, is very aromatic and pungent. It is the darkest, being a dark yellow brown.

Japan ginger contains much altered compound starch.

Calcutta ginger contains spherical starch, which resembles in outline that of wheat, and many cork cells.

Exhausted ginger contains few yellow brown resin cells, and altered starch.

Decorticated ginger looses much of the pale yellow oil, which is replaced by brown or reddish resin, which is almost insoluble.

Hydrastis has vessels, spherical starch, 3 different marked tracheids. By adding to the powder sulphuric acid, a separation of acicular crystals may be seen taking place from the parenchyma cells. If the powder is first moistened with water before adding concentrated sulphuric acid various shaped crystals will form. It has cork with red brown walls.

Serpentaria has cells of the phloem and xylem lignified bast, parenchyma with yellow brown contents. The parenchyma of the cortex and pith has spherical 2 to 4 compound starch. It contains a few non-glandular trichomes from the stem which are characteristic.

Berberis contains cells of the phloem and xylem, variously marked vessels, medullary rays 2 to 9 cells wide and contains starch, and the bast, cork and parenchyma have yellow brown contents.

Mezereum has typical bast, uneven, irregular bent, non-lignified and colorless—a few ellipsoidal 1 to 4 compound starch grains. The cork is yellow brown. The medullary ray cells contain starch. The parenchyma and collenchyma contain a yellow green content. It has no xylem cells.

Pareira contains xylem and phloem cells. The tracheae have slit like pores. The starch is oblong 2 to 4 compound. The bast is slightly lignified. Some of the wood parenchyma are lignified. There are few cork cells, and black in color. The wood cells are thick walled.

B. Drugs containing little or no starch.

a. Those containing calcium oxalate.

1. In rosette form.

Anisum has non-glandular 1 celled trichomes with centrifugal projections, aleurone grains, narrow brown epidermal cells and oil.

Italian anise has no trichomes but has many yellow brown cells. Adulterated with Conium, which, when an alkali is added, produces a red brown col-

or; then if picric acid T. S. is added a granular precipitate forms. (Test for Conium).

Foeniculum contains aleurone, yellow brown cells with crystals, short, brown and very narrow epidermal cells with oil, which separates out when the powder is placed in chloral T. S.

Calendula has long narrow epidermal cells, with wavy walls, many oil globules and chromoplasts, which turn brown when sulphuric acid is added, spherical pollen with centrifugal projections and 3 pored, "T" shaped non-glandular 1 to 2 stalked trichomes. The powder when moistened with water gives a straw color.

2. In monolithic prisms.

a. Thick walled parenchyma, spiral and annular tracheae, oil cells.

1. Rich green color with hydrochloric acid.

Aurantii Amari Cortex.

Aurantii Dulcis Cortex.

2. No color change with hydrochloric acid.

Limonis Cortex contains small tabular epidermal cells, hypoderm cells with chromoplasts, thin walled parenchyma, schizogenous and lysigenous oil cavities and parenchyma with granular protoplasm.

b. Containing no calcium oxalate.

1. With bast, stone or wood cells.

A. With pigment cells.

Sinapis Nigra has beaker shaped stone cells of unequal height, brown pigment cells which are blue with ferric chloride T. S.—mucilage in the epidermal cells and no starch.

Foengreek contains large thick walled, pored stone with triangular lumens—endosperm cells and yellowish embryo cells with aleurone, oil and mucilage.

Linum has epidermal cells with mucilage, a few bast which are yellow to red brown—pigment cells, blue with ferric chloride T. S. and no starch. The endosperm and embryo cells contain much oil and many aleurone.

Cydonium has a mucilaginous epidermus, aleurone grains, fixed oil, bast with brown contents, "tube cells," starch and brown pigment cells.

B. No pigment cells present.

Sinapis Alba with a mucilaginous epidermis, typical "beaker shaped stone," of unequal height, aleurone, fixed oil, obliterated cells, and cells with yellow contents and proteids.

Pepo has few starch (in outer epidermis and endosperm only), characteristic ellipsoidal, thick walled, porous cells of the seed coat with yellow contents, oil, protein, aleurone, small elongated palisade, of the cotyledon and lignified outer epidermis.

Colocynthis has typical stone, some are lignified, a few fixed oil and aleurone, epidermis reticulately thickened and the seed coat cells have a yellow brown content.

2. Containing few or no bast, wood or stone.

A. With pollen.

Arnica Flores contains 3 pored spherical pollen, corolla fragments and 3 kinds of glandular trichomes, 1 to 5 celled, or 2 celled with pores in the dividing walls and 1 to 5 celled single or double stalk, and a 1 to 2 celled head. The achene cells have dark brown walls and content.

Sambucus—elder flower has the typical pollen and flower cells.

Matricaria with the triangular, 3 pored, spinose pollen, fragments of the corolla, glandular 8 celled trichomes, a small amount of tannin and achene cells with scalariform perforations and papillated stigma cells.

Calendula. See previous description.

Crocus mounts are deep red in glycerin, a few nearly smooth spherical pollen, fragments are blue with sulphuric acid, changing to violet and finally to deep wine color (differing from Capsicum). The stigma cells have cylindrical papillae.

Carthamus—or American saffron with sulphuric acid is yellow and in 2 minutes becomes colorless, and on standing some time becomes deep wine color similar to Crocus.

Anthemis contains a few thick walled trichomes, some are sessile glandular, the cuticle spinos-reticulate, cells with chloroplasts or yellow coloring, the pollen is spherical; the bast and large parenchyma cells contain yellow brown resin and tannin.

B. Containing no pollen.

Aspidium is diagnosed by the starch, oil, lignified hypoderm, tracheae, endoderm and the powder turning violet with sulphuric acid. D. marginalis has 6 F. V. B's. D. filix-mas has 7 to 9 F. V. B's.

3. Containing no phloem or xylem cells.

Lycopodium, see pages on natural powders.

Lupulinum, see pages on natural powders.

Cambogia, see pages on non-cellular drugs.

2. Drugs containing few or no plant tissues or cells.

A. Those which give on heating the odor of sulphur dioxide.

Sulphur Lotum, forms in chain like masses in glycerin mounts.

Sulphur Precipitation forms irregular rounded masses in glycerin mounts.

B. Those which give no sulphur dioxide odor on heating.

1. Nearly colorless in glycerin mounts.

Mastiche forms in transparent masses, soluble in ether, nearly so in alcohol, which solution is acid to litmus.

2. Yellowish in glycerin mounts.

Scammoniae Radix, oil, irregular masses, see non-cellular drugs.

Resina, see pages on non-cellular drugs.

a. Translucent or transparent.

Sandarac soluble in alcohol gives a colorless solution, and on heating in water will not run together as Resina.

Aloe in glycerin mounts is soluble, leaving acicular crystals, see pages on non-cellular drugs.

b. Opaque.

Cambogia.

DRUGS WHEN FRESHLY POWDERED WHICH ARE OF A BROWNISH COLOR

This group includes Roots, Rhizomes, Barks, a few Flowers, Fruits or Seeds and a few non-cellular drugs. It is divided into two parts: 1st. Those drugs which contain cells of the phloem or xylem; 2nd. Those drugs which contain few or no cells of the phloem or xylem.

A. Those with starch and calcium oxalate crystals.

a. Calcium oxalate in rosette crystals.

1. With oil, resin or tannin.

Belladonnae Radix contains sphenoidal micro-crystal cells, starch 2 to 6 compound, a few wood cells, many tracheae and tracheids, cork cells, no tannin. The crystal cells with alkalies or choral T. S. appear as gray black cells.

Gossypii Cortex has diagnostic bast, which is long, narrow, thick walled and lignified. The parenchyma contains a red-yellow pigment and tannin. Secretion cells contain a reddish brown amorphous content. The cork cells are thin walled and reddish brown. Starch grains are 1 to 4 compound.

Juglans contains a few crystal bast, wide and very long. The stone cells are elongated; parenchyma cells have oil, tannin or a purple brown content.

Aralia has spherical starch, tracheae with spiral, scalariform, reticulate or pored markings, the wood cells are very long and brownish cork.

Rubus has diagnostic long thin walled lignified bast, parenchyma with crystals, oil or tannin, crystal fibers, a few stone, non-glandular trichomes, and starch single or compound.

Stillingia has diagnostic irregular tuberculated long, narrow, thick walled slightly lignified bast which swells in alkalies, red brown cork, yellow brown pigment cells, cells with starch, oil or resin.

Canella, or white cinnamon, has single, or 2 to 3 compound starch, stone cells with branching pores, a few bast, (if any they are lignified), many large oil cells with suberized walls.

Euonymus has a typical, long, thin porous walled, non-lignified bast, which is associated with long cells, which are curved—starch, cork, yellow brown secretion cells; the contents are soluble in ether

or chloroform. The stem bark contains chlorophyll and epidermal cells with stomata.

Rumex has elongated starch, stone, light brown cork, fragments in aqueous mounts turn red with alkalies (chrysophanic acid), fragments are blue green with ferric chloride T. S. (tannin).

Rheum, see previous description.

2. Containing little or no resin or tannin.

Althaea is diagnosed by the thick porous walled, slightly lignified bast, many large mucilage cells, a few wood cells, cambium cells with brown walls and starch centric cleft.

3. Containing oil secretion reservoirs.

Caryophyllus contains small spindle bast, crystal fibers, tetrahedral 3 pored pollen, tracheae, much oil and little tannin. The stem cells have thick walled irregularly isodiametric stone.

Pimenta contains a few monoclinic prisms, starch 1, 2 or 3 compound with a centric cleft, the stone cells have thick walls and have nearly colorless contents, oil secretion cells with wine colored contents. Reddish brown tannin masses turn greenish with ferric chloride T. S., or with ferric ammonium sulphate solution. The trichomes are non-glandular, and thick walled. Cells of the embryo.

4. Containing no oil secretion reservoirs.

Galla.

Geranium contains starch, tracheae, parenchyma with tannin, many resin cells, rosette calcium oxalate crystals. The pith contains starch. Cells of the rhizome are found.

b. Monoclinic or pyramid crystals and bast, wood or stone.

1. Containing crystal fibers.

Krameria has a diagnostic bast, whose walls are wavy, non-lignified with sharp ends. The starch is 2 to 5 compound, the wood cells are thick and porous; many red brown walled cells contain a yellow content which turns red with alkalies, little cork.

Juniperus contains crystal stone or stone with yellow brown walls, schizogenous oil cells, brown pigment cells, aleurone, thick walled epidermal cells

with a brown content, hypoderm cells and collenchyma with a brown amorphous content.

A. Red with alkalies.

Cascara is characterized by having bast, stone, crystal fibers with monoclinic prisms, starch, parenchyma with yellowish content, which turns red with alkalies, few cork cells, the walls of which are yellow or brown, some may contain amorphous content. Medullary ray cells are red with alkalies, a few rosette crystals, many dark brown collenchyma cells.

Frangula (no stone).

B. Not red with alkalies.

Quercus has crystal bast, long thin walled lignified bast, thick walled stone, parenchyma with grey yellow tannin and no cork.

Prunus Virginiana contains starch, monoclinic and rosette crystals, bast and thick walled stone, chlorophyll bearing cells, little or no cork.

Calamus, see page on rhizomes; try the vanillin test.

Ulmus.

Hamamelidis Cortex has crystal bast, thick walled lignified porous stone, parenchyma containing red brown tannin, or very small starch or crystals, medullary ray cells and sieve.

Pulvis Aromaticus contains cells of Saigon Cinnamon, Jamaica Ginger, Cardamom Seed and Myristica.

Pulvis Glycyrrhizae Compositus contains cells of glycyrrhiza, senna, sulphur granules and sugar crystals.

Pulvis Ipecacuanhae et Opii contains milk sugar crystals, cells of ipecac (starch and tracheids, intermediary fibers) and matrix of the Opium (poppy capsule cells). See pages on these drugs.

c. Containing calcium oxalate in raphides.

Veratrum viride.

Convallaria contains 2 to 4 compound starch, tracheae, endoderm cells with thick inner walls, bast long thin walled and simple pores.

Cinnamomum—Saigon has bast in groups of 2 to 20, thick walled and slightly lignified, the starch is single or compound, the stone has colorless walls, irregular in outline, and contains air or red brown

amorphous content, the parenchyma has tannin and oil and many large oil secretion cells.
Cassia cinnamon contains few cork cells, many bast and stone and large oil cells.
Ceylon cinnamon contains single or compound starch, attenuated ended bast and stone.
Cassia buds have thick walled, curved non-glandular trichomes, the bast are broad and blunt.
Sarsaparilla is diagnosed by the endoderm which has yellow or red porous walls. It contains bast, tracheae. No stone (from the stem).
Hydrangea.
Cypripedium has single or compound starch, tracheae with many pores, thin walled endoderm cells, and bast.

d. Containing sphenoidal micro-crystal cells.
Belladonnae Radix.
Cinchona has diagnostic spindle shaped bast with yellow walls, parenchyma with a few starch (2 to 5 compound), or with red brown tannin masses. Grahe's test.—Heat a little of the powder in a test tube or on the dished slide, and the brighter red colored the distillate is, on the sides of the test tube, or on the cover glass, the more alkaloids the drug contains. Calisaya cinchona contains many starch, and the distillate is a purple red. See pages on barks.

B. Containing no calcium oxalate.
Strophanthus has characteristic trichomes, which are bent, non-glandular, thick walled at the base and thin walled at the apex, and somewhat lignified. The powder in chloral T. S. or alkalies shows much oil, aleurone and a little starch. The endosperm is greenish with sulphuric acid, and no crystals of calcium oxalate or stone cells are found.

1. Containing few or no stone, bast or wood cells.

a. Containing tracheae.
Convallaria.
Sarsaparilla.
Zingiber.
Guarana—See non-cellular drugs.
Sumbul contains oil resin secretion cells, parenchyma with starch, fragments of the yellow epidermis, red brown oleoresin cells, sieve, laticiferous, a few simple pored wood cells, some resin secretion cells with a bluish brown content.

Valeriana contains stone, many 2 to 4 compound centric cleft starch, the wood cells are thin walled porous and lignified, a few root hairs, some cork and cells of the phloem and xylem.

Cacao is diagnosed by the inner epidermal cells, cells with proteins, oil, starch (which may be swollen and coheres with water and heat), cells with a brown purple content, cacao butter in prisms or needle crystals, mucilage cells and isodiametric stone.

Cacao shells have characteristic brown red pigment cells, mucilage, hairs, few or no starch hexagonal epidermal cells and isodiametric stone.

Tonka cotyledons contain parenchyma with starch, large irregular aleurone, fixed oil, stone cells of the seed coat which are characteristic and thick walled, porous, with a red brown content.

Cypripedium.

Leptandra contains parenchyma with starch or a blackish colored pigment, thick porous walled wood cells, pigment cells (with chloral T. S. turn pink), and epidermal cells with thick walls.

Spigelia contains few tracheae, and bast which is non-lignified, some starch, red brown epidermal cells, and root cells.

Coffee has the spindle shaped twin stone cells. The endocarp cells are brownish and contain tannin, starch, oil, and aleurone. Fragments should float on water.

Piper has the typical "horse shoe shaped" stone, parenchyma with the red brown amorphous masses, oil resin and starch. Some stone cells contain yellow brown tannin masses.

Colchici Cormus is diagnosed by the 2 to 6 compound starch, thin walled epidermal cells, brownish red concentric F. V. B's. and some of the fragments are yellow, changing to red with sulphuric acid.

Aconitum has the diagnostic starch 1 to 5 compound, stone cells, yellow brown cork, parenchyma and cambium cells with starch.

Physostigma has the diagnostic reniform starch, test tube shaped palisade and non-lignified red brown epidermal cells.

Colchici Semen contains stone cells with a pigment which is soluble in alkalies and the tannin reaction

is obtained with the pigment. The endocarp cells are thin walled and the parenchyma and palisade contain starch.

Myristica contains many oil cells, starch 2 to 20 compound and aleurone. Mount the powder in olive or other fixed oil, and the oil will separate out in rod like crystals. Limed nutmegs with sulphuric acid causes a separation of the calcium sulphate in needle like crystals. Mace starch is red with iodine T. S.

Opium, see non-cellular drugs.

Podophyllum contains 2 to 6 compound starch, a few rosette or raphide crystals. The cork is red brown —many resin and rhizome cells.

Chenopodium has elongated colorless thin walled cells, reniform seeds with brown pigments, starch and aleurone.

Cimicifuga contains starch, thick walled periderm cells with red brown contents.

Apocynum has 2 different kinds of bast, laticiferous with yellowish content, much starch. A. androsaemifolium contains stone.

Guarana.

Methysticum.

Scopola contains sphenoidal micro-crystal cells, tracheae and parenchyma with starch.

Senega has thick walled, pored non-lignified bast, medullary rays slightly lignified.

Lappa contains parenchyma with inulin, and very few tracheae.

Chirata has spiral, scalariform and pored tracheae, narrow long thick walled, pores slightly lignified bast, pith cells slightly lignified. Collenchyma cells with brown yellow resin and tannin, pollen may be present (elliptical, oblong and prickly).

b. Containing no tracheae.

Cinchona.

Cinnamomum.

Sassafras Cortex contains parenchyma with yellow red tannin masses, starch 2 to 4 compound, a few bast, which are irregular with pointed ends, and little or no cork.

Ulmus contains thin walled non-lignified bast, a few crystal bast, with monoclinic crystals, spherical starch and many mucilage cells, and no cork.

B. Containing few or no starch grains.
a. Containing calcium oxalate.
1. In rosettes.
a. Crystals in aleurone grains.
Anisum.
Carum has many buff colored cells, the inner epidermis is yellowish brown, many oil cells, the endosperm contains aleurone, each aleurone grain contains one rosette calcium oxalate crystal. The inner epidermis is composed of yellow brown polygonal cells.
Coriandrum has narrow yellow inner epidermal cells, much fixed oil, aleurone, curved bast which is thick walled and lignified.
Foeniculum.
Conium.
b. No crystals in the few, or no aleurone grains.
1. With pollen.
Arnica Flores.
Caryophyllus.
Pyrethrum Flores.
2. Few or no pollen.
Cusso has diagnostic trichomes, non-glandular 1 celled, curved thick walled, and glandular trichomes with 2 to 3 celled stalk and 1 celled head, or the head with 1 to 2 cells. The bast are long thick walled, lignified and with oblique pores. The pith parenchyma is lignified. Pollen is ellipsoidal and 3 pored.
Brayera, the N. F. term for cusso.
Viburnum Opules contains no cells of the xylem but crystal fibers with monoclinic prisms, few or no stone, many thick wavy walled lignified bast, parenchyma and medullary rays contain starch and yellow brown amorphous substance and a few sieve cells.
Viburnum Prunifolium contains rosette crystals in crystal fibers, few or no bast. The stone is irregularly thickened and lignified, with light yellow walls. The cork is brown red.
b. In monoclinic crystals or prisms.
Vanilla contains many minute black seeds (clear with KOH to see structure), unicellular trichomes with heavy balsamic content. Fragments deep red with phloroglucin T. S., and sulphuric acid (due to vanil-

lin). Tonka an adulterant contains much starch.

Xanthoxylum contains starch, light yellow oil secretion reservoirs, also some colorless oil cells. The cork is thick walled and lignified, a few bast fibers (swells in chloral T. S.), medullary ray cells, some flat rod shaped crystals (not calcium oxalate).

Quercus.

Macis is characterized by the amylodextrin, (red with iodine T. S.) Santonica has spherical pollen, smooth walled, 3 pored, diagnostic trichomes—fragments in alcohol mounts and heated, then KOH T. S. added giving a red color.

1. Containing many stone.

Cubeba contains diagnostic stone, (some have yellow content; fragments are wine colored with sulphuric acid. Cells with tannin, and starch, and suberized walled oil cells.

Staphisagria.

Pyrethrum (Root) contains parenchyma with inulin, stone with yellow brown contents, resin canals, resin in the medullary rays, a few 1 celled root hairs. The wood cells are porous and light yellow. The cork is brownish. Yellow brown amorphous masses of resin and volatile oil.

2. Containing no stone cells.

Gentiana contains no stone, bast, wood or tannin, but a few tracheae, many intermediary fibers which are non-lignified and have pored walls. It contains much collenchyma, some fixed oil, cambium cells and the cork is brownish.

Opium.

Taraxacum contains parenchyma with inulin, many sieve, laticiferous with yellow brown contents, some tracheae, non-lignified intermediary fibers and pith. The wood cells are yellow and porous.

Chicory has parenchyma with inulin, branching laticiferous, elongated parenchyma, tracheids with sharp pointed ends. Fragments sink and color water yellowish (difference from coffee).

Triticum with tracheae, lignified epidermal and hypodermal cells, bast are long and thick walled and parenchyma cells contain a soluble carbohydrate.

2. Few or no cells of the phloem or xylem.

Ergota contains parenchyma filled with hyphae and a

small amount of fixed oil (use chloral T. S.). Fragments are colored red-violet with chloral T. S. and sulphuric acid, a blood red color is produced when the sulphuric acid is added. The color is soluble in alkalies with a violet colored solution.

Goa powder.

Chrysarobin, see non-cellular drugs.

A. Containing oil.

Asafoetida, see non-cellular drugs.

B. Without oil.

1. Opaque in glycerin mounts.

Aloe-Socratine, see non-cellular drugs.

Benzonium.

Elaterinum.

Lactucarium.

Gambir.

2. Translucent in glycerin mounts.

Aloe.

DRUGS WHEN FRESHLY POWDERED WHICH ARE OF A REDDISH COLOR

1. Drugs containing starch.

Quillaja has many monoclinic crystals of calcium oxalate in the bast. The bast is irregular, branching and lignified. It also contains sieve cells, crystal cells, a little starch and a few or no stone. Some cells have clumps of saponin.

Sanguinaria has the epidermal, parenchyma and laticiferous cells with fixed oil, tannin, starch and red secretions, many secretion cells, tracheae, laticiferous with resin, cells of a rhizome or root. Fresh sections when placed in glycerin for 24 hours show aggregate crystals.

2. Drugs containing little or no starch.

A. Containing stone cells.

Capsicum has a striated epidermis, diagnostic stone (2 kinds of stone), secretion cells with oil, or resin, or chromoplasts. Yellowish red oil cells and starch are from the unripe fruits. It may contain a few trichomes—Capsicutin, a constituent, imparts its taste to water when in 1 in 11,000,000 dilutions.

Illicium (star anise) contains diagnostic palisade like stone, isodiametric stone with branching pores, also isodiametric stone from the seed. The parenchyma contains aleurone and oil.

Cydonium.

Rhus Glabra contains non-glandular trichomes filled with air, multicellular trichomes with wine colored pigment in the head, fixed oil in the embryo, resin cells, gallic and tannic acids.

Rosa canina fructus contains non-glandular one celled, tapering towards the base and apex, thick walled trichomes. Stone and parenchyma have red contents, rosette crystals, and elongated cells with brown contents. Embryo cells contain oil and aleurone.

Carbo Ligni is black-red in mounts. Debris of xylem cells.

B. Drugs containing no stone.

1. With wood cells.

Haematoxylon contains tracheids, resin, tannin, crystal wood, monoclinic crystals. The drug colors

water reddish. The wood cells are long and thin walled . To a water mount add alkalies and a purple red solution results, which on standing becomes deep red. When ammonia is added to a water mount on standing several hours a crystalline precipitate is formed.

Santalum Rubrum contains tracheids, crystal fibers, and imparts no color to water, but colors alcohol and alkalies. The wood cells are thin walled and long. The medullary ray cells may contain tannin.

2. Containing no wood cells.

Opium.

Kino.

Lupulinum.

Zea contains spiral and annular tracheae, non-glandular trichomes in cells of two parallel rows and spinose pollen. Fragments are purple to grey and thread like. The cell contents are tannin and resin. It imparts to water, alcohol or acids a yellow red color, and with alkalies a green color.

Rosa Gallica has a spherical pollen. The fragments are deep blue with iron solutions. Fragments in water mounts are rose or brownish red. The upper epidermis has trichomes, the lower epidermal cells are rectangular. All cells contain tannin, oil and a purple red content, which colors water and alcohol; then if sulphuric acid is added, it becomes yellow but if alkalies are added it becomes brown.

Rosa centifolia has elliptical pollen. Fragments with chloral T. S. are pink.

Crocus.

DRUGS WHEN FRESHLY POWDERED WHICH ARE OF A WHITISH APPEARANCE

1. Drugs containing more or less characteristic starch.
   A. Those with few plant cells.
      a. Containing only "unaltered starch."
         The various starches, see page on starches.
      b. Containing both altered and unaltered starch.
         Dextrin is sticky and slowly soluble in water, blue to brown with iodine T. S. Dextrin is composed of chiefly altered starch.
         Sago has characteristic small horny grains, separating in water.
   B. Those with many plant parts.
      Quillaja.
      Calamus.
      Ulmus.
      Nux Vomica contains diagnostic lignified 1 celled trichomes. Each epidermal cell produces a trichome. The endosperm cells have fixed oil and aleurone, and turn blue with potassium dichromate T. S. and sulphuric acid. There is much starch in the thick walled parenchyma cells. Fragments with alcohol and sulphuric acid show crystals.
      Bryonia with tracheae, containing yellow contents. The cork has thin yellow walls, which when sulphuric acid is added turn a purple red color. The parenchyma contains 2 to 6 compound centric cleft starch.
      Orris root contains starch which is diagnostic, tracheae, raphides and prisms of calcium oxalate, secretion cells and parenchyma with yellow contents, but no cork.
      Oat meal and rolled oats contain starch, aleurone, epidermal cells and trichomes which are diagnostic.
      Barley flour contains diagnostic starch, trichomes and cells of the caryopsis with latticed walls, short curved blunt ended bast and parenchyma with yellow or green content. The endosperm cells contain aleurone grains.
      The endosperm cells contain aleurone grains.
      Rice flour is diagnosed by the characteristic starch grains.
      Corn meal has many parenchyma cells with oil and the characteristic starch. The cells of the epicarp have wavy porous walls.

Corn bran contains less starch and oil, but more cells of the hull.

Wheat flour agglutinates with water, contains some seed cells, little tissue and the diagnostic wheat starch.

Wheat middlings contain the endocarp cells with aleurone, embryo cells, fixed oil, trichomes and starch.

Oat meal contains aleurone, epidermal cells, characteristic starch and trichomes, and elongated wavy walled cells of the pericarp.

2. Those containing no starch.

A. With calcium oxalate.

Scilla has thin walled epidermal cells with stomata, raphides of calcium oxalate (the largest raphides found in any U. S. P. drug), tracheae, mucilage and F. V. B. tissue. Fragments in alcohol or glycerin show sugar crystals.

Amygdala Amara.

Amygdala Dulcis.—The structures of the two almonds are practically the same. They contain rectangular stone, cubical endosperm cells, brown hypoderm cells. The cotyledons contain aleurone and fixed oil. The inner epidermis has a brown content, and some of the epidermal cells are lignified.

B. Containing no calicum oxalate.

Nux Vomica.

C. Containing few or no plant parts.

a. Soluble in water.

Acacia.

Saccharum, rhombic prisms. Soluble in alcohol, glycerin and water, insoluble in chloroform and ether.

b. Swell in water.

Tragacantha.

c. Insoluble in water but soluble in alcohol.

Camphora.—See U. S. P. for solutions. Sublime a small amount on the dish slide and examine cover glass for crystals. Evaporate some of the alcoholic solution and note shape of crystals. Add a little of the powder to vanillin; then add sulphuric acid; a yellow color forms, changing to red, then to violet and finally to blue.

d. Insoluble in water glycerine or alcohol.

Saccharum lactis occurs in small irregular dry crystals. With sulphuric acid a reddish color is produced.

Talc occurs in irregular masses or crystals, lustrous,

soapy feel. Usually the crystals are in broken masses.

1. Soluble in acetic acid with effervescence.

Calcii Carbonas Praecipitatus: When a hot solution tion of ammonium oxalate is added to an acetic acid mount of the drug, crystals of calcium oxalate precipitate out. In glycerine the drug appears as aggregates or cubical irregular sized crystals.

Creta Praeparata, when treated as above, yields triangular and cubical crystals of various sizes.

Barium carbonate. When sulphuric acid is added to a glycerin mount, barium sulphate is precipitated as very small crystals.

2. No effervescence with, but soluble in acetic acid.

Magnesii Oxidum Ponderosum appears in glycerin mounts as rounded masses. To a citric acid mount add an excess of ammonia water, then sodium phosplate T. S., and stir with a glass rod. Triangular or tetrahedral crystals will form.

Magnesii-oxidum resembles in glycerin mounts the heavy magnesia, but it is larger and more transparent. With the above test crystals of ammonium magnesium phosphates are formed, in the shape of stars.

3. Insoluble in acetic acid but soluble in nitric acid.

Calcium Phosphate. Soluble in nitric acid and when a drop of ammonium molybdate T. S. is added, and stirring crystals with a glass rod, small yellow diamond shaped crystals are formed which are permanent in glycerin.

Barium Sulphate in glycerin mounts appears as irregular shaped crystals of various sizes. Try the green flame test with your platinum needle.

Terra Alba.—Irregular masses.

Terra Silicea Purificata. See your U. S. P.

Diatomaceous earths, characteristic diatoms.

The word is derived from Baktron—a rod or staff—and Logos—a discourse. Bacteriology may be defined as "The branch of biology that deals with bacteria."

In Biblical times, the belief prevailed that all epidemics were plagues. The Biblical use of the word plague is like its medieval use, viz: To denote a pestilence or epidemic, rather than its specific use, as in bubonic plague.

The term plague was used to designate the ten plagues of the Bible, one of which probably was the bubonic plague. For in Samuel we read that the Philistines were smitten with emerods, or tumors in their secret parts. The Ark was sent to Gath, then to Ekron; but the plague still followed. There seems to have been a plague of mice, spoken of as "mice that mar the land;" or in the revised version "in the midst of the land thereof mice were brought forth, and there was a great and deadly destruction in the city;" and again, "their land swarmed with mice." The Ark was returned to Bethshemesk, having golden images of the tumors and of the mice; but still the plague continued, and more than 50,000 persons perished. It has taken over three thousand years to prove that the rat flea and its specific bacillus as agents, spread disease.

The first notice of prophylaxis appears when Moses swung censers through the plague smitten camp. It was probably through the Mosaic law of cleanliness that the whole race of people at that time were not wiped out. Moses also caused large bonfires to be built in the camp, to drive away pestilence. Sulphur was used by the shepherds to destroy lice on sheep.

The ancients believed that diseases originated in the soil, water or air. That epidemics were caused by divine wrath—supernatural causes; the changing of climates, stars, hence "dog days," (see pages on Rabies), decaying bodies on the battlefields, noxious gases, fogs, rains and many other things.

In 640 B. C., Hippocrates, the father of medicine, believed that plagues were caused by the changing of seasons.

In 610 B. C., Aximander of Melitis said "Many animals originate spontaneously and develop in a day."

In 450 B. C., Empidocles of Agrigentun claimed that all living things originated spontaneously.

Aristotle in 383 B. C. said that only some things may originate spontaneously.

In 50 B. C., Ovid gave instructions for creating bees in carcasses of horses by saying some magic words. As late as

1542, Cardau stated that water created fish and animals by fermentation.

Varo in 25 B. C. said "There are minute invisible animalicules in the swamp air, which when breathed will cause disease." This was probably the disease now known as Malaria —from the Italian meaning mal—bad—and aria—air. We now know that this disease comes not from the air but from a parasite carried by the mosquito. It was named Malaria by Forti in 1712 and was proven to be caused by mosquitoes by Lancasi in 1717. The disease was known to Hippocrates as "swamp fever;" and a complete description was given by Celeus A. D. 25.

Thorac in 1260 A. D. used wine to cover and dress wounds, which as he thought, when they became sore, came from infections from the air.

In 1542 Cardau claimed he could develop spontaneously fish and animals by fermentation. Later he claimed that only the lower animals as lice, and the lower plants as sea weed, were developed spontaneously.

In 1600 it was proved that diseases could be and were carried by persons traveling by boat, and spread from seaport to seaport.

The first microscope was produced by Hooks in 1658, and perfected by the "father of bacteriology," Leenhouck, in 1662. This was the first microscope capable of revealing micro-organism. Leenhouck was the first to see micro-organism, plant cells—differentiated mono from di-cotyledons and placed the use of the microscope on a scientific basis. He also proved Harvy's law, "Omne vivum ex ovo, or vivo," the circulation of blood; disproved spontaneous generation such as fleas originating spontaneously from dust—that eels did not come from dew; and that lice and worms did not originate in pigeon dung; and many other discoveries, as yeast, in 1660.

Kercher in 1664 suggested that diseases might be due to micro-organism which under certain conditions developed spontaneously. It was about this time that the first micro-organisms were discovered in the mouth and in stagnant water.

In 1667 Spallanzi boiled fermentable liquids in a tube, shaped like a retort, sealed the ends and found no fermentation took place.

Redi proved in 1686 that by screening meal, maggots did not come from putrefying meal but were caused by flies.

Plenciz found certain bodies in disease and claimed in

732, that all diseases were caused by these bodies (micro-organisms).

Jenner in 1768 discovered vaccination.

Noah Webster as late as 1799 claimed that the wrath of God caused an agitation of the elements and that epidemics always followed earthquakes. He believed in spontaneous generation.

Ehrenburg in 1826 isolated micro-organisms from water and dust and in 1833 formed his classification of the shapes of bacteria, as bacterium, vibrio, spirillum and spirochaeta.

That yeast caused fermentation and produced alcohol and CO2, was proven in 1837 by Schwan. He also proved that organisms causing decay come from the air.

Schultz in 1836 prevented fermentation and decay in liquids, by heating in a flask with a long contorted neck and filling the contortions with sulphuric acid.

In 1840 Henle taught that diseases were carried by the air, and that all contagion was by contact.

Devine in 1850 discovered rod shaped organisms in splenic fever.

Pollander in 1855 discovered the cause of anthrax. About this time the relation of micro-organisms to disease and fermentation was investigated.

Thorac in 1860 taught that wound infections were from the air, and used boiling oil to pour over the wounds. At that time it was taught, that the more pus in a wound, the better the healing. Haller states that all diseases are caused by micro-organisms.

In 1866 Rindfleish and Waldeyer proved wound infections due to micro-organisms, and pus not of benefit to normal healings but due to infection.

Pasteur, a French chemist, was born in 1882, educated at Jena, later graduating from four colleges. He was a foremost teacher in chemistry and bacteriology, and was one of the greatest discoveries in these sciences. Among his discoveries were—the action of crystals to polarized light; laevo-rotatory; molecule dissymmetry; and especially the part played by micro-organisms in fermentation and decomposition. He introduced a successful treatment of the silk worm disease. He achieved great success in his efforts to check hydrophobia and rabies. To aid him in his researches, an institute was opened in Paris and another in Lille, France. These were followed by similar institutions in all parts of the world. Due to overwork, he became paralyzed in 1868 and died in Paris, September 28, 1895.

From 1880 to 1890 great advances were made in the study of bacteriology. Suitable apparatus and culture media were discovered. Bacterial stains were put to practical use. Previous to this time, liquid culture media were used; but this method had many drawbacks. Gelatin and agar were added to form solid culture media.

Lister was born in 1827 and died February 12, 1912. He was a Professor of Surgery in Edinburgh, Scotland, and was surgeon to the Queen and King Edward. He was a celebrated writer and made many discoveries, among which were the coagulation of blood, inflammation, and taught from the first that pus indicated an infection; and that pus was due to decomposition in the blood and tissues; that the infection came from the air. He taught the importance of absolute cleanliness in operations. Pasteur's belief in micro-organisms caused Lister to apply principles of antiseptics, thus revolutionizing surgery, by which millions of lives have been saved. Lister used Phenol as his antiseptic; but this is not satisfactory now. He received many honors, the highest being that of Peer, in 1897.

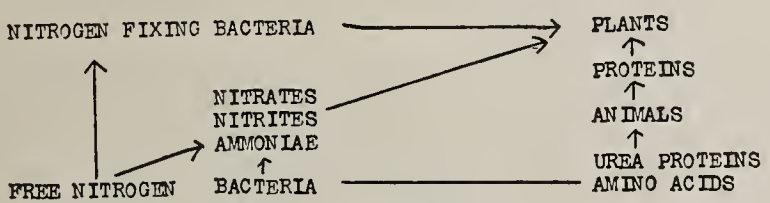
Robert Koch in 1879 investigated the causes of consumption and in 1882 discovered the tubercle bacillus. In 1884 he was placed in charge of the expedition to Egypt and India to investigate the causes of cholera and discovered the comma bacillus. He announced a remedy for the cure of consumption, which he called the tuberculins. In this investigation he found a method of making a solid culture media.

As late as 1890 some professors still held to the pus theory, and opposed the micro-organism theory.

From 1890 to 1897 rapid strides were made in bacteriology, serums, bacterial vaccines, and various kinds of immunity were discovered. The organism causing Rabies was discovered by Leteve, of Pittsburgh; the tuberculins by Koch; opsonins by Wright; typhoid vaccine; starters for butter making; cheese; cider and wine starter; flavoring; by the use of micro-organisms. The use of the nitrogen-fixing organisms in agriculture; the use of micro-organisms in paper making; leather tanning; destruction of animal pests, and many others.

The nitrogen fixing organisms convert ammonia into nitrogen and acids. Some plants take nitrogen from the air, others from the nodules, as in the Leguminosae, as seen in the clovers, etc. The nodules are filled with rod shaped bacteria

—Rhizobium radiciola. The organisms converting ammonia are the Aztobacter and others. Nitrogen may be replaced by crop rotation as in clover and grains. In this way the exhausted soil is refilled with the nitrification organisms—by the breaking down of dead animal and vegetable bodies by the nitrosome organisms, into simple compounds. The denitrification, or reducing organisms, reduces nitrates to nitrites and ammonia, etc.—setting free the nitrogen as in proteins, decompositions, etc. These cultures of nitrifying bacteria may be obtained from the Government or from private laboratories, and are used by moistening the grain with the cultures and drying before planting; or sown or sprinkled over the ground. They have been used very successfully.



BACTERIA IN AGRICULTURE.

In tanning leather and removing the hair from hides, cultures of the Proteus bacteria are used; and for drenching, cultures of Bacillus erodiens and furfuri are used—shortening the time for curing.

Tobacco curing has become a science. The brown color due to enzymes has often been spoiled by saprophytic fungus, Botrytis, causing the "pole burn." This has been practically eliminated by drying in specially prepared rooms. The tobacco after being dried is placed in piles, which undergo heating and fermentation. In this process the loss of nicotine is about 28%. Various organisms take an active part in this fermentation. Many manufacturers add to the tobacco, cultures of certain organisms which give the tobacco a better aroma than that which is ripened under ordinary conditions. The cultures are a secret, but evidently are of the Proteus, Subtilis and Mycoides groups. These cultures are sprayed on the tobacco leaves before being placed in piles. Spraying uncured leaves with a mixture of water, rum and molasses mixed with an old infusion of tobacco (which contains the specific organism which will give the specific flavor) is called "petuning." Havana tobacco cured in the United States will not have the Havana tobacco flavor, as there are some organisms indigenous to Havana which must enter into the process.

The same is true of Java and other tobaccos. A large field awaits an investigator in this as well as all other industrial lines of bacteriology.

In butter making instead of using starters (a small quantity of cream that has ripened), pure cultures of the bacteria which cause the ripening of cream are used. In this way uniform butter is always obtained. These cultures consist of about 95% lactic acid organisms (Streptococcus lacticus and others). In cheese making the cultures are also used. Here bacteria and various molds form the cultures.

Preserving food: Drying vegetables by exposing to the sun or artificial heat, or smoking as in meat, causes the moisture to be expelled. This renders the organic substances inadaptable for bacterial growth and as long as they are kept dry will be preserved.

In the smoking of meats, the creosote and other products in the burning wood act as an antiseptic. But not all bcateria are killed by this process; those of consumption, swine erysipelas in pickled pork and other organisms retain their viability for a long time. A strong solution of salt used in pickling, inhibits the growth on account of the solution being of such a greater density than the bacterial cell protoplasm. It also has a tendency to extract the water from the cell protoplasm. The preserving power of vinegar is due to the acetic acid.

All preservatives are dangerous and need not be used if the food is wholesome and not spoiled. There is no need to use preservatives in catsup if the tomatoes are good. Some manufacturers avoid the stating of preservatives on the label, by using an artificial cinnamic aldehyde, (which is supposed to come from the cinnamon used as one of the spices).

Eggs treated with Sodium Silicate (1 in 10 parts of water), makes the shell impervious to bacteria and delays the decay. No egg has been found which is free from bacteria. An examination of 2,500 eggs showed that 8% had infected yolk—9% of all unbroken shell eggs contained bacteria—of these 11% contained cocci, 28% rod shaped organisms, and 61% spirilla, probably from the hen ovaries—practically all of the broken shell eggs contained various organisms—3% of the eggs examined were leakers. There were fewer bacteria found in clean eggs than dirty eggs. In 1916 an average of 210 eggs per person were consumed in the United States, totaling a value of $300,000,000.

In a fresh egg the yolk is firm, and as it ages the yolk membrane breaks down and mixes with the white and forms weak eggs, which usually have an odor.

Candled eggs show the fresh eggs as having a firm central yolk having only a slight movement, on aging the yolk becomes more opaque.

In fruits, such as apples, the decay may be delayed by wiping the skins dry and polishing with a clean cloth—All bruising must be avoided.

Low temperature, 4.44 degrees C., or below, as in cold storage, checks some of the bacterial growth. See pages on Bacteria Found in Milk. Some bacteria multiply at or near freezing point. Foods usually keep well in cold storages but must be used soon after taking them out, as they spoil quickly—many foods spoil in 24 hours or less.

Heating as in preserving and hermetically sealing, kills nearly all micro-organisms; but some spores resist heat and remain (anaerobic) growing, or multiplying slowly. These last cause the spoiling of the fruits by generating gases which cause the swelling or buldging of tin cans, or bursting of glass jars. Corn must be heated to 121 degrees C. to prevent spoiling. Fruit frequently spoils on account of the cans not being sealed tight, imperfect soldering, too thin tin plates, etc.

Vinegar is formed from cider at 18 to 24 degrees C., and it may be hastened by adding "mother of vinegar," which is composed of mycoderma aceti and other micro-organisms. This process must have an abundance of oxygen. Hence the containers are filled only ⅔ full. The sourness is sometimes lost by the presence of Bacillus xylinum, which in the presence of oxygen splits up the acetic acid, and other compounds formed in the cider. This is prevented by stoppering the containers and shutting off the oxygen supply.

Sourkraut is a lactic acid fermentation produced by various micro-organisms, when air is partly excluded from the fermenting mass.

In baking there are two kinds of fermentation produced, a normal and an abnormal. Many times the dough is spoiled by the potato bacillus—Bacillus mesentericus, which is highly resistant to heat. The dough is raised by gas being generated by yeasts and probably some of the coli group.

Starters are sour dough containing great numbers of the gas producing micro-organisms and yeasts imbedded in the dough.

Top yeasts (see page on Yeasts) are dried, or pressed, or in fluid form. These yeasts produce carbon dioxide and alcohol; and the bacteria which are mixed with the yeasts produce carbon dioxide and hydrogen. Pure cultures of yeast produce a bread containing but little butyric and acetic acids, which makes the bread more digestible. When bread molds, penicillum, rhizopus and aspergillus are the most common causes. Pellagra is said to be caused by molded or spoiled grain.

Cellulose in decaying plants is destroyed rapidly by micro-organisms as stated by "Omelianski." Cellulose weighing 3.47 grains after disintegration weighed 0.12 grains showing decomposition of 3.34 grains, into 2.24 grains of fatty acids—0.97 grains into carbon dioxide and 0.010 grains into hydrogen and oxygen.

BUY FOODS FROM DEALERS WHO USE SCREENS PROTECTING THE FOOD FROM DUST AND FLIES, AND FROM THE HANDLING BY PURCHASERS.

Street dust and flies are especially dangerous. Fruit bought from a vender's stand on the street showed the following: Pieces of prunes, beans and rice, human hair, cat fur, cotton and wool fibres, straw and bran fragments, insect wings and legs, cigar and cigarette ashes and a great number of micro-organism, many of which were pathogenic. Many infectious diseases have been traced to such beginnings.

An editorial in the Journal of the American Medical Association some time ago, said under the heading, "Asepsis and the Dish Towel": "In restaurants the white enamel and plate glass from the outward view appear clean.—The dish towel at the waitress' belt is used to wipe and polish the glasses of the departing guest and the glasses are replaced on the table for the next guest, and the table and plate wiped with the same cloth. Which probably at one time (and one time only when freshly washed and boiled) was clean, used in soiled hands, until bacterially grimy, carried on the arm of the waiter, and used for many purposes—belongs to the dark ages, before men began to look through the microscope and think in terms of microscopical cleanliness. The day is coming when Bridget will be compelled to sterilize her dish cloth." Laws are now in force requiring all persons who handle food in public places to be examined every month and in some states every six months for communicable diseases. Laws are being made for inspections to ban the dirty, germ carrying waiters and replace them with clean, healthy ones. Typhoid, consumption, syphilis and other diseases have been traced to these sources. You as pharmacists can set a good example—approaching the practice of the laboratory where glasses are washed and rinsed in boiling water and placed to drain. You can use this method with your fountain glassware, wash in hot soap suds, rinse in boiling water, turn upside down to drain—the result will be clean sterile glasses, shining and polished.

A law passed by the Pennsylvania legislature, which went into effect January 1, 1916, required all persons handling food in public places to be examined every six months for communicable diseases and to have a certificate stating they are free from communicable disease. A heavy penalty is provided for not complying with this rule.

A law in Pittsburgh, of 1917, states:

Department of Health.

Notice:

The sale of soda waters, lemonades, orangeades, pops, non-alcoholic drinks, ice creams, sundaes, water ices and all similar beverages and refreshments is strictly prohibited in the City of Pittsburgh, unless same is served in sterilized glasses, cups, saucers or containers. All spoons or utensils used in eating or drinking must be thoroughly cleansed and sterilized, before being used by another person.

By sterilization is meant the immersion in boiling water, or the use of steam or heat which will bring the above mentioned glasses, cups, saucers, spoons, utensils and containers to a temperature of 212 degrees Fahrenheit. (100 degrees C.)

Any person who shall fail, neglect or refuse to comply with this order shall be subject to a fine, or imprisonment, or both.

Dr. J. C. McNeil,
Superintendent, Bureau of Food Inspection.
Dr. R. G. Burns,
Act. Director, Department of Public Health.

A much easier method—and complying with the law—is to use the paper cups and dishes which are made to fit special holders. The outlay at first is but little more than that of the glasses; but by using the paper dishes and cups, the soda trade will increase, as people will see that you are up to date in your methods, and that they will have dishes and cups that have not been used, and that are clean. The spoons also must be sterilized.

Some states require vaccination and tests for typhoid fever in milk handlers.

Samples of food purchased in Chicago showed that, in one sample of water-cress obtained from a glass bowl containing water in a free lunch counter in a saloon there were many pathogenic organisms. In 22 out of 29 samples of green foods, coli were found. Of samples from 12 stores classed as clean, 11 showed coli. From 10 samples of stores classed as fair, 6 showed coli; and from 6 samples from dirty stores, 5 showed coli. Streptococci, mold and other organisms were found in many of the specimens. Twenty-eight of the 29 specimens showed the presence of gas forming organisms. A bacterial count of the watercress was made and totaled 26,000,000 colonies of micro-organisms.

Hand to mouth infection: Long before anything was

known about bacteria, it was the custom of decent people to wash their hands before eating. Even the most ignorant mother sends her children from the table when they come with dirty hands, and tells them it is not necessary for them to eat the proverbial peck of dirt. The surgeon spends much time in cleansing his hands so that they will not carry infection to the wounds, and he also wears rubber gloves. Bacteria may be carried into the mouth without harm which would endanger life if introduced into the body through wounds. In the army the soldier is instructed to wash his hands after visiting the toilet and before eating; and we may take the health of the soldier as an example for us to follow.

The hand shake may be very dangerous as infection is carried by the hands The old lady who wore a ring, having a fine poisoned lancet, furnishes a good illustration. When she shook hands, the lancet was pressed and the friend was inoculated with the deadly poison. This is true in every day life. Noses are blown into handkerchiefs, which are handled; some of the nasal secretions (full of micro-organisms) cling to the hands, and remain viable when dry. Then hands are shaken with good friends, transmitting the micro-organisms along with the hand shake; and in many cases infection has been traced to this cause. It perhaps would be better to use the Chinese method of greeting—to shake our own hands.

If saliva was colored a bright red we would be amazed at the color of our fingers, for our fingers are carried to our nose and mouth many times a day, there to implant the bacteria of diseases which other careless people have spread about them. If hands and fingers were kept away from the nose and mouth there would be much less sickness.

Objects touched by fingers moistened with saliva, as turning the pages in books, telephone directories, saliva moistened money, street car transfers and hands offered for shaking after being used to cover the droplets of a cough, are a few of the many ways for acquiring micro-organisms fresh from the respiratory tract. Palmer has found that in an ordinary day's life, there were 119 chances of acquiring infection, such as hand contact with door knobs, etc. Putting coins or lead pencils into the mouth is a reprehensible habit. Many organisms may be harmless on the hands but pathogenic when carried into the mouth. The typhoid carrier who is not cleanly in his personal habits, and who carried a dish of soup from kitchen to parlor, with his thumb being washed by the soup is especially dangerous; this occurs frequently.

The soiling of the hands is impossible to avoid, but the swallowing of micro-organisms from one's hands is a matter of individual control.

Many states prohibit the use of common towels, prohibiting the placing, keeping or use of common towels in any public lavatory.

The general conclusion of most practical observers has been that the route of hand infection is the great pathway of infection, and is responsible for perhaps 90% of all contact infection, medical or surgical, except the venereal diseases. While the handshake does not rank with kissing in directness, it is not confined, like kissing, to the exchange of nasal or oral discharges, but includes the discharges of the bowel and bladder. Moreover, the hands are so constantly infected and handshaking is so constantly repeated that, in the long run, Hill says, it probably eclipses the relatively less common and less promiscuous kissing in its sum total damage.

During an examination of food handlers in New York City in 1918, over 44,000 were examined; these included cooks, waitresses, kitchen employes and bakers, both male and female. Of this number, 25 cases of consumption, 41 cases of syphilis. Of the 211 waitresses examined, 66% showed anemia, 62% showed varicose veins, 18% had menstrual disorders, 4 had active syphilis. The cooks showed 32 with heart disease, 161 with digestive disorders. There were 38 cases of bronchial diseases among the bakers, 30 had heart disease, and 33 anemia.

In another examination of the food handlers in New York City, out of 1,980 persons examined there were 390 females. 80.2% of the persons examined were between 20 and 44 years of age. 81% were restaurant and hotel workers. 16% were confectioners and bakers. Of the 81%, 44% were waiters and waitresses, nearly 20% were cooks and 9% dish washers, 32% of the males and 13% of the females were free from disease. Among the others were 25 cases of consumption, 51 cases of syphilis and 6 of gonorrhea. 370 cases of anemia, 112 cases of eye disease, 124 of heart disease, 64 with diseases of the arteries, 237 with varicose veins, 110 with colds and rhinitis, 25 cases of chronic bronchitis, 104 with pulmonary emphysema, 208 cases of pyorrhea alveolaris, 288 cases of dental caries, 202 cases pharyngitis, 62 cases of hernia and 133 with flat feet.

The Portland, Oregon, Board of Health has adopted a system of cards for food handlers. A store whose sanitary

inspection rates 90% or more is given a card rated as "A." The store whose rating is from 80 to 90% is given a card "B." And the card "C" is given to the stores rating under 80%. These cards must be displayed so that all customers may see them. A frequent inspection is made to see that the cards are displayed. A large improvement has been made in all the stores as the merchants are anxious to get the card, rating them as "A."

Dutchess County, N. Y., in 1918 spent $412,000 for sickness that could have been prevented, to say nothing of invalidism, bereavement and loss of productive power. In 1,600 cases of serious illness, it was estimated that 9,000 working days were lost, and 13,700 school days were lost, these two items costing $25,000. (Dutchess County Health Association).

The cost of illness in the state of Illinois for the year ending July 1, 1918, reached the total $154,881,665, according to a statement by the director of the state department of health, Dr. Drake. Tuberculosis cost $114,905,500; pneumonia, $30,909,360; typhoid fever, $3,006,900; malaria, $2,660,860; diphtheria, $1,156,625; whooping cough, $735,220; small pox, $675,600; infantile paralysis, $461,600; maesles, $456,020; epidemic spinal meningitis, $425,700, and scarlet fever, $388,300.

The Bulletin of the Indiana State Board of Health says: "The greater proportion of people are born healthy and their way of living makes them sick. The people of America are only 50% efficient because of ill health and disease. Apparently our population is 100,000,000; efficiently it is only 50,000,000. This is the result of wrong feeding, cranky immoderation, not enough air and sunshine, impure and insufficient water drinking; alcohol, caffein and nicotine addiction, and our awful and absurd use of drugs and patent medicines."

The United States Census Bureau for 1910 shows there were nearly 190,000 persons in hospitals for the insane and nearly 21,000 persons in institutions for the feeble-minded, and the cost was more than $160,000,000. There are two insane and four feeble-minded persons in every 1,000 of the population. Of the 85,000 persons in almshouses, over 42,000 have some form of insanity; of over 137,000 persons in prisons, jails, etc., at least 30,000 were mentally diseased.

The first selective draft rejected 24 out of every 1,000 on account of being mentally diseased.

EPILEPSY IN THE UNITED STATES

Only 3.5% of the epileptics are cared for in stitutions. 200,000 persons suffer from epilepsy. Only 13 states have colonies for the care of epileptics, and they care for only 7,000 patients. One in every three epileptics cannot write; one in every four can write and read; only 40 per thousand have a common school education; thirty-two per thousand have attended night school; and four per thousand have attended college. The Massachusetts Commission of Mental Diseases (July, 1918) find that epileptics have abnormalities in the pineal gland and pituitary body. Mental deterioration is in direct proportion to the frequency and severity of the convulsions. Proper food, regulated work and routine life lessen the convulsions and postpone death.

ANTISEPTICS

Antiseptics kill all micro-organisms but not necessarily all spores.

Antiseptics are classified as those which cause destruction by:

1. Oxidation as ozone, chlorinated bases, potassium permanganate, etc.
2. Ionic poison with coagulation as metallic salts.
3. Coagulation and poisoning, which are not ionic as phenol, etc.
4. By emulsoid action, through the brownian movement and absorption.

Germicides kill all life and spores.

DISINFECTANTS

Disinfection kills all life; and the term is usually applied to the treating of rooms contaminated by infectious diseases. Disinfectants are classified as:

1. Potent as bacteria destroyers.
2. Soluble in water and readily penetrate or permeate solid organic substances.
3. Comparatively non-toxic to man externally or internally.
4. Minimum albumen coagulating power and penetrating substances readily.
5. Comparatively cheap and usable by those of average intelligence.

STERILIZATION

Sterilization means the destroying of all life.

Sterilization is accomplished by:

1. Heat, physical means, electricity, sunlight, filtration, etc. By heat.
   - A. The naked flames, as the bunsen burner kills all life.
   - B. Dry heat, as the material placed in the drying oven or dry sterilizer, (substances not injured by heat, as glassware).
   - C. Steam heat or moist heat.
     - a. Under pressure as in the autoclave, using 15 to 20 lbs. pressure for 15 to 40 minutes a day for 3 days.
     - b. Without pressure as in rice or double cookers, or Arnold sterilizer, for 15 to 40 minutes a day for 3 days.
2. Chemical means as by using phenol, creosote, bichloride, etc.
3. Mechanical means, grinding, filtration, centrifugalization, etc.

Sterilization may be divided into two kinds.

1. Discontinuous or repeated sterilization is employed where high heat is not permissible, and consists in heating the substance for 15 to 40 minutes a day for 3 days; this repeated sterilization is for the purpose of killing all organisms and spores. Practically all organisms are killed at 100 degrees C. at the first heating. The materials are then set aside in a warm place for 24 hours, so that any organisms or spores which may have survived the first heating may grow; a second heating kills all life; but in order to be absolutely positive that all life has been destroyed a third heating or sterilization is necessary.

2. Continuous sterilization is used for material which is not destroyed by high heat, such as glassware. This is accomplished by one sterilization.

Culture media for growing micro-organisms are prepared by discontinuous or repeated sterilization; by sterilizing in the autoclave (care must be taken to not close the escape valve until steam pressure has been developed or the steam will 15 lbs. pressure for 15 to 30 minutes a day for 3 days. The not penetrate all parts of the material to be sterilized) at

nedia for yeasts and molds should be slightly acid, but for bacteria they should be slightly alkaline. Media containing carbohydrates which are destroyed by high temperatures, must be discontinuously sterilized in a double boiler as a rice cooker or Arnold sterilizer.

The duration of bacterial life after the first 24 hours is reduced, and all are usually killed by prolonged sterilization, chemicals, filtration, grinding and sunlight.

Nearly all of the known basteria are killed after boiling in water for one hour, but some of the spores may resist 3 hours at 100 degrees C. Moist heat, which is more penetrating than dry heat, at 130 degrees C. for 1 to 2 hours usually kills all bacteria and spores; there are some exceptions as the hay bacillus.

Water at 200 degrees C. for 10 minutes usually kills all typhoid bacilli.

Pasteurization, another form of sterilization, (see pages on Bacteria in Milk), is a partial sterilization, killing most of the pathogenic bacteria. A temperature of 60 degrees C. for 30 minutes without any appreciable physical change in the substances, is usually applied to milk.

Chemicals as peroxide of hydrogen destroy some pus forming organisms; but it is very mild and loses its strength rapidly on account of its chemical composition. Its deturgent action is very good, when applied to wounds containing pus and dead epithelial and blood cells, as is seen in the "foaming."

Bichloride is hindered by albumen.

Formaldehyde vapors, or spray or 300 gms. in 1 L. of water is usually sufficient for each 1,000 cubic feet of space to be disinfected.

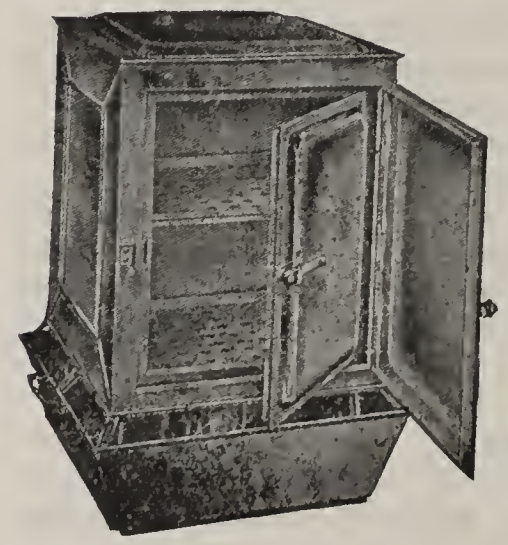
Sulphur burned in a warm room and over water vapors requires 300 gms. to every 1,000 cubic feet.

Phenol loses its germicidal power in oily solutions.

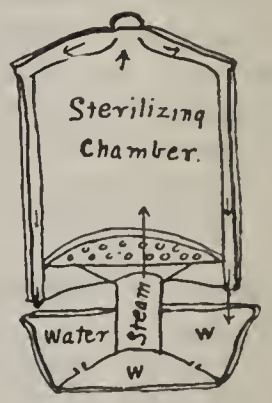
The U. S. P. Tinctura Iodi is one of the best for external applications.

Phenol is very liable to cause gangrene. But by Dr. Perez's method of applying pure liquid crystal phenol to the point of application with a swab or brush there is little danger. After the burn appears as a white spot, the part is to be touched with a swab soaked in hydrogen peroxide; this will prevent any danger of ulcers forming, or gangrene. If the spot remains white, it means that the tissue is healthy; but if the tissue is diseased, the spot will darken untli almost black; and the nearer the diseased tissue is to the spot touch-

ed the darker the spot will become. This method has been used with great success in producing local anesthesia. Boils and carbuncles after being treated, may be incised; and this may be repeated every 24 hours, until all the hardness disappears and true healing takes place; which is indicated by the unmixed white color at the point treated with the phenol and hydrogen peroxide.

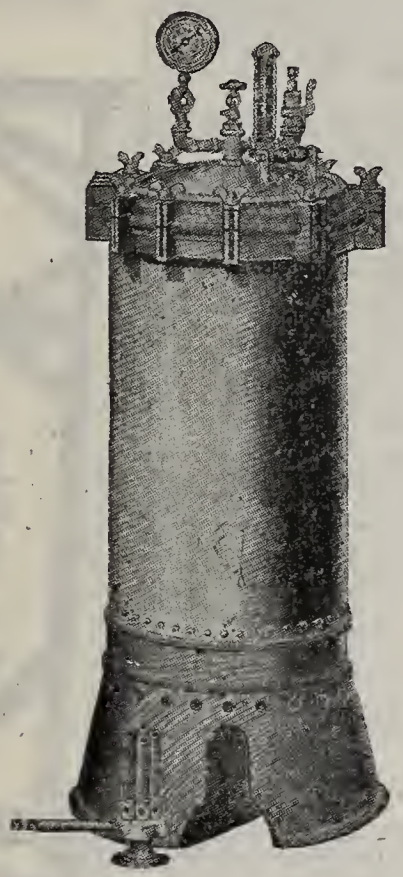


Arnold Steam Sterilizer

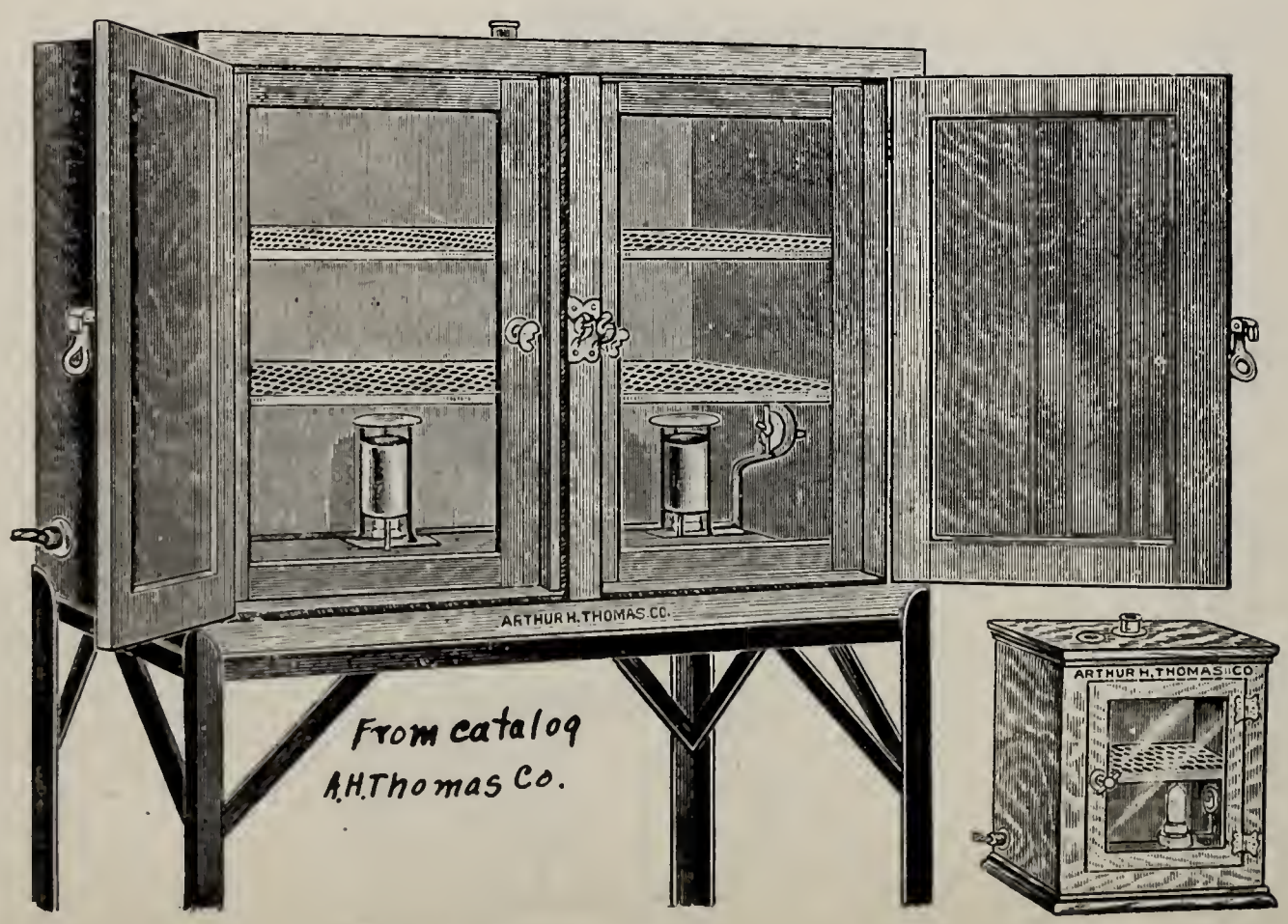


SECTIONAL VIEW.
ARNOLD STERILIZER.

A cheap and effective sterilizer may be made from any wooden box, preferably of tin or zinc, as a tin cake box with a tight fitting lid, and by placing perforated shelves at intervals. Liberating formaldehyde gas for one hour usually suffices. The formaldehyde-potassium permanganate solutions are more rapid. The box must be absolutely tight, all cracks and openings must be stoppered. This is readily accomplished by using adhesive paper or tape.



Autoclav/



Electric Incubators

ESTING DISINFECTANTS

ple means of testing disinfectants or anti-
a number of dilutions of the substance to
tubes plainly marked as to the strength.
ed in cultures of Bacillus prodigiosus and
diluted solutions for a certain number of
ced in test tubes (properly numbered) of
solution. If the B. prodigiosus has not been
nfectant or antiseptic, the Dunham's solu-
d red in 24 hours.
Coefficient Method of Testing Disinfectants.
Walker method of obtaining the "Hygenic
l coefficient." This is for the purpose of
rmicidal power of the disinfectant or anti-
phenol.
of different known strengths of phenol so-
8 test tubes; place these in the water bath
20 degrees C. 8 other test tubes are placed
h, each containing 5 mils of different dilu-
fectant to be tested. 1-10 of a mil of a 24
of Bacillus typhosis (supplied by the Hy-
) is placed in each test tube. At the end
utes and up to 15 minutes a platinum loop
23 wire and 4 mm. in diameter) full of the
ach test tube is inoculated into a properly
containing 10 mils of standard broth cul-
inoculated tubes are incubated at 37 degrees
nd then examined for growth.
representing the degree of dilution of the
h of the disinfectant that kills within 2½
ed by the figure representing the degree of
is determined.
are noted, comparative strength of phenol
vn strength disinfectant selected, and the
weakest strength of the phenol control that
utes. The same is done for the weakest
lls in 15 minutes. The mean of the two is
For example, the weakest solution of pheno
½ minutes was 1 to 80; and the weakest solu-
in 15 minutes was 1 in 110; and the weakest
unknown strength disinfectant which killed
was 1 in 375; and the weakest solution which
kills in 15 minutes was 1 in 650; then 375 divided by 80, plus
650 divided by 110 equals 4.69 plus 5.91, or 10.60. This num-

—to replace deleted lines—

the dilution of the weakest strength of phenol which killed In this time, the result is added to the figure obtained by dividing the dilution of the weakest strength of the unknown which killed in 15 minutes by the dilution of the weakest strength of phenol which killed in 15 minutes. This sum is then divided by two, the result being the Phenol Coefficient.

(For complete details of this list, see Bulletin Hygenic Laboratory No. 82)

Example.

Kilied in 2 1-2 minutes. Killed in 15 minutes.

$$\frac{\frac{\text{Unknown}}{\text{Phenol}} \text{ plus } \frac{\text{Unknown}}{\text{Phenol}}}{2} = \text{The Phenol Coefficient.}$$

OR

$$\frac{\frac{1:375}{1:80} \text{ plus } \frac{1:650}{1:100} - 5:30}{2}$$

To find the cost ratio oi a disinfectant compared wifh phenol.

$$\frac{\text{Cost of disinfectant}}{\text{Cost of phenol}} \div \frac{\text{Disinfectant coefficient}}{\text{—Phenol coefficient}} = \text{the Cost Ratio.}$$

ber divided by 2 equals 5.30 or the disinfectant value compared with that of phenol, or 5.3 times the value of phenol.

A number of conditions modify this test—the organism acted upon, the number of organisms, the amount of organic matter added, the strength of the dilutions, temperature, etc.

The phenol coefficient is the killing power of the substance compared with that of phenol, upon the same bacteria and under the same conditions.

This test without organic matter yields higher results as Phenol Liquefactum U. S. P. without organic matter the coefficient has 177 and with organic matter 176. Crude phenol is 2.75 and 2.63. Cresol 2.90 and 1.75, and Liquor Cresolis Compositus is 3, and with organic compounds 1.87.

ESTING DISINFECTANTS

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a number of dilutions of the substance to
tubes plainly marked as to the strength.
ed in cultures of Bacillus prodigiosus and
diluted solutions for a certain number of
ced in test tubes (properly numbered) of sterile Dunham's solution. If the B. prodigiosus has not been killed by the disinfectant or antiseptic, the Dunham's solution will be colored red in 24 hours.

The Phenol Coefficient Method of Testing Disinfectants.

The Rideal-Walker method of obtaining the "Hygenic Laboratory phenol coefficient." This is for the purpose of comparing the germicidal power of the disinfectant or antiseptic with that phenol.

Place 5 mils of different known strengths of phenol solutions in each of 8 test tubes; place these in the water bath, which is kept at 20 degrees C. 8 other test tubes are placed in the water bath, each containing 5 mils of different dilutions of the disinfectant to be tested. 1-10 of a mil of a 24 hour old culture of Bacillus typhosis (supplied by the Hygenic Laboratory) is placed in each test tube. At the end of every 2½ minutes and up to 15 minutes a platinum loop (made from No. 23 wire and 4 mm. in diameter) full of the mixtures from each test tube is inoculated into a properly labeled test tube containing 10 mils of standard broth culture media. The inoculated tubes are incubated at 37 degrees C. for 48 hours and then examined for growth.

The figure representing the degree of dilution of the weakest strength of the disinfectant that kills within 2½ minutes is divided by the figure representing the degree of phenol coefficient is determined.

The results are noted, comparative strength of phenol, and the unknown strength disinfectant selected, and the dilution of the weakest strength of the phenol control that kills in 15 minutes. The same is done for the weakest strength that kills in 15 minutes. The mean of the two is the coefficient. For example, the weakest solution of phenol which kills in 2½ minutes was 1 to 80; and the weakest solution which kills in 15 minutes was 1 in 110; and the weakest solution of the unknown strength disinfectant which killed in 2½ minutes was 1 in 375; and the weakest solution which kills in 15 minutes was 1 in 650; then 375 divided by 80, plus 650 divided by 110 equals 4.69 plus 5.91, or 10.60. This num-

er divided by 2 equals 5.30 or the disinfectant value compared with that of phenol, or 5.3 times the value of phenol.

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THE USE OF BACTERIOLOGY TO THE PHARMACIST

The "up to date" pharmacist must realize the relation bacteria bear to the human activities, and fully understand the significance of these organisms in pharmaceutical practice. He must know the substances liable to be attacked, how to prevent the attacks upon pharmaceutical substances, and know the changes of bacterial deterioration, and the results of these changes, when introduced into the human body.

The progressive pharmacist is qualified to sterilize substances as required by the various Pharmacopoeias of the different countries. He must sterilize all substances to be used on abraded surfaces. He will be required to standardize disinfectants and antiseptics and to be able to detect yeasts, molds and other organisms developing in syrups, tinctures, etc., and to test milk, and water for pathogenic bacteria, and to make total counts and isolations. He must be able to prepare antiseptics, in fact he must be an aid or assistant to the physician in all laboratory work, such as analyzing urine, feces, and sputum, make tests for diphtheria, typhoid fever, and the gonococcus, make blood counts and bacterial vaccines.

The pharmacist must sterilize all solutions or medicinal substances which will be used for intravenous and hypodermic injections; and all those to be applied to cut, bruised or abrased surfaces, wounds, sores or ulcers; and in general all those medicinal substances to be applied to the broken skin or mucous membranes as enemas, bladder irregations, eye and mouth washes; all ointments must be sterilized and placed only in collapsible tubes—Do not use ointment pots which may be returned for refilling in a soiled and contaminated condition. Diseases have been contracted in this way.

HINTS TO THE UP-TO-DATE PHARMACIST

KEEP YOUR STORE CLEAN. Keep it sanitary. Give your customers a good example to follow.

Do not refill old bottles, or ointment pots. Who knows whether or not the bottle has been standing for some time in a room with a patient suffering from a contagious disease, or the ointment pot has been handled by a patient with repulsive and disgusting sores? Mere washing will not prevent contagion.

Keep the rims of the dispensing bottles clean—free from dust. A ring may be made of celluloid or cardboard and placed on the cork, which will prevent dust from contaminating the lip of the bottle and also the fluid poured from the bottle

Use paper towels, not linen. Paper towels are less expensive, more sanitary and cannot be used as long as the linen, hence they will be more hygienic. Paper towels can be used to clean spatulas, wipe mortars, etc. Paper towels will not be dropped from the prescription counter to the floor, tramped on and then picked up and used to wipe a container in which medicines are to be mixed or placed.

Keep your hands clean, not alone while filling prescriptions, but at all times. Do not be afraid of water and soap. Your customers will appreciate clean hands.

Do not roll pills between the fingers or hands; you have proper appliances for this purpose.

Handle medicinal substances as little as possible.

Count pills, tablets, etc., directly from the container into the box. Never use your hands in which to count the pills, etc. Keep the pill and powder boxes away from all dirt and dust and keep them clean. Do not blow dust out of the boxes; you will contaminate the boxes with organisms from your mouth, which may not be pathogenic to you, but may be very pathogenic to others.

Use tissue or oiled paper when necessary, but not the hands.

Do not refill old bottles, ointment pots, pill or powder boxes; use new ones; the patient appreciates clean looking, neat packages. Use collapsible tubes for ointment. These cannot be refilled.

You as pharmacists must set the example in cleanliness to the layman.

Bottles and glassware are sterilized by washing in water, (all wash water to be distilled), rinsed with 2 to 5%

NaOH solution; then neutralized in 1% HCl, washed well with water, plugged with cotton, wrapped in paper and heated in the hot air sterilizer for 2 hours at 170 degrees C. (U. S. P. method is heating from 120 to 160 degrees C. for 15 minutes to one hour), or by steam pressure at 130 degrees C. for 30 to 60 minutes. They may also be sterilized by placing in a current of steam for 30 minutes, or by boiling in a 1 in 1,000 sodium bicarbonate solution for 15 minutes.

Tin containers and mortars are to be sterilized the same as bottles, or alcohol may be added and set on fire. Fire destroys all life.

Corks and stoppers are to be sterilized the same as bottles—after sterilization they are to be stored in wide mouth, sterile, cotton stoppered bottles, and handled with sterile forceps only.

Surgical supplies as cotton and bandages are to be wrapped in cheese cloth and placed in the steam sterilizer at 115 degrees C. for one hour, or in the dry oven at 160 degrees C. for one hour.

Surgical instruments are sterilized by boiling in water, to which has been added a little sodium bicarbonate, which prevents tarnishing. Or 1% NaOH may be used.

Sterilization of surgical instruments is best occomplished by boiling in liquid petrolatum for 10 minutes at 150° C. This will prevent the dulling of the cutting edge and also cover the instruments with a thin film of oil, preventing rust to a large extent.

Syringes may be sterilized by boiling in water; but if the syringe is to be used for taking specimens of blood, no soda or NaOH should be added, as this might lead to the laking of the blood, and it also interferes with the smooth operation of the syringe when dry.

Rubber will not permit frequent and prolonged boiling, so formaldehyde vapors are used. Use rubber stoppers only for sterile bottles.

Medicinal substances as tooth powders, dusting powders, etc., are sterilized by dry heat at 70 degrees C. for 3 or 4 hours.

Solutions of boric and tannic acids, waters and liquids intended for wound irrigation, salt solutions, oils, etc., for hypodermic use, in general any substance which will not stand prolonged heating as the sugars, gelatin, etc., may be sterilized for 5 minutes a day in steam for a number of days.

Solutions readily decomposed by heat must be filtered through porcelain filters.

Alkaloids and glucosides in solutions, tinctures and fluid extracts, should be filtered and placed in sealed containers, as yellow or brown colored ampules, and sterilized at 60 degrees C. ½ hour a day for 3 to 6 days. These usually keep as long as unopened, but as soon as opened, oxygen and micro-organisms gain entrance and spoiling rapidly results. The ampule is the best method, as the solutions keep longer, and are always ready for use, and less danger of infection exists than when tablets and other solutions are used.

All solutions of atropine, cocaine, hyoscyamus, scopola, physostigma, ergot, digitalis and strophanthus are acted upon by light and must be preserved in sealed amber ampules.

For morphine solutions, the solution is placed in sterile bottles (sterilized as under bottles) loosely stoppered, and sterilized in live steam for 30 minutes. A yellow solution results. The morphine is then recrystallized by evaporating in vacuum over KOH and no color results when it is mixed in fresh sterile water.

Adrenalin chloride solutions are sterilized by placing them in ampules and boiling in water for 30 minutes, or in steam for 15 minutes a day for 3 days.

Bicarbonate solutions may be sterilized by placing the bicarbonate solution in citrate of magnesium bottles, add some carbon dioxide gas and several drops of phenolpthalein solution and sterilize in steam sterilizer. When sterilized and cold, open the bottle and shake; if it is pink in color on account of the change to carbonate, reject it.

Generally the substance which is not readily decomposed by heat may be sterilized by steam at 115 to 120 degrees C. for 30 to 60 minutes.

The following table is given by Lesude, in the Schweizer Apotheker Zeitung:

1. Sterilize in the autoclave at 115 degrees for 15 to 20 minutes:

Adrenalin, sodium benzoate, brucine sulphate; iron, guaiacol and sodium cacodylates; caffeine; sodium cinnamate; cocaine hydrochloride; salts of codeine; creosote; eucaine; sodium formate; gelatin (twice or thrice); guaiacol; heroine; holocaine; acetic acid; magnesium sulphate, salts of mercury with mineral acids; salts of morphine; novocaine hydrochloride, salts of pilocarpine; salts of quinine; non-phosphatic sera; strychnine sulphate; stovaine.

2. Sterilize at 100 degrees:

Salts of aconitine; adrenalin; alypin; apomorphine hydrochloride; salts of atropine; brucine; sodium cantharidate;

cantharidin; cinnamic acid; colchicine; duboisine sulphate emetine hydrochloride; galyl; potassium, sodium and iron glycerophosphate; hysocyamine hydrochloride; ludyl; methylene blue; narceine hydrochloride; nirvanin; salts of physostigmine; quinine cacodylate; scopolamine hydrobromide.

3. Sterilizable by Tyndallization (four to five times at 60 to 70 degrees):

Aristol; chloral; curare; ergotinine; hectine; hectargyre lecithin; sodium nucleinate; oils.

4. Sterilizable by filtration through a Chamberland filter

Calcium glycerophosphate; organic extracts; isotonic salt solution; therapeutic sera; tuberculin; vaccine; mineral waters; yeast.

5. To be prepared with aseptic precautions:

Crystalline aconitine; arsacetin; collargol; cholesterin all colloids; cryogenin; crystalline digitalin; electrargol and all electric colloids; ichthyol; most mercury salts with organic acids; medicated oils; protargol; salvarsan; neosalvarsan.

GENERAL DISINFECTANTS

A disinfectant is a substance which destroys the cause infection. The most simple and best disinfectants are nlight (the blue rays are destructive to organisms, while e yellow rays cause them to thrive), and heat. Micro-ganisms are rapidly destroyed when in a dry state by sun-ght; but on account of some of the micro-organisms cling-g to dust particles or organic matter, the destruction is de-yed; and some cases boiling for a short time fails to kill.

Halogens and their compounds are all active disinfect-nts; but bromine and fluorine are too corrosive for general se. Chlorine is used in the form of the gas (see pages on ater) and in the form of hypochlorites which yield chlor-e.

Iodine in solution especially as the official tincture, al-ough an irritant, is one of the best and most frequently nown antiseptics and disinfectants. For the best effects, the kin must be thoroughly dry before applying. It attacks acteria less energetically after coming in contact with the kin. Iodine in 1 to 2,000 kills staphylococci in one hour but ill not injure body tissues. It is destructive to some of ne larger parasites infesting the skin. Iodine combinations s iodoform, thymol iodide are mild in their action; iodoform not actively disinfecting, but when applied to most wounds, decomposes slowly, liberating active iodine. It may be bsorbed from open wounds and cause poisoning—disturbing ne nervous central system and in a few days producing rest-essness, anesthesia, unconsciousness, or frequently produc-ng ulcers which are hard to heal. Iodoform has been of alue in suppositories for consumption of the colon.

Chloride of lime is a mixture containing hypochlorites which yield free chlorine and is a common household disin-ectant and bleaching agent.

Antiseptic Hypochlorite Solution. This is composed of ry sodium carbonate 140 grams dissolved in 10 L., tap water nd 200 grams of bleaching powder containing 24 to 28% vailable chlorine, shaken. And after it stands 30 minutes, he clear liquid is siphoned off and 40 grams boric acid is dded, and the liquid is ready for use.

Neutral Hypochlorite solution is the above solution made eutral.

These solutions may be made by adding chlorine gas, 1.8 grams to the liter of a solution containing 14 grams odium carbonate.

These solutions are unstable and an excess of boric acid

should be avoided, and the solutions should not be kept mo
than a week.

Chloramin-T, or sodium toluene sulphon chloramin c
chlorazene can be used in stronger solutions than the hypc
chlorites. It is more stable, exerts more prolonged antisepti
action, and is less irritating and non-toxic, but has littl
solvent action on necrosed tissue.

Dichloramin-T, or sodium toluene sulphon dichlorami
is very valuable when dissolved in oil mediums for sprays
or poured into deep wounds. It is suitable for cases requi
ing prolonged antiseptic treatment.

Chlorcosane is a heavy oil prepared from paraffi
wax by replacing part of the hydrogen in the compounds b
chlorine. This is a bland, tasteless oil similar to castor o
in viscosity. The chlorin is attached to the carbon and i
inert, like the chlorin in common salt. This oil will hol
about 10% dichloramin-T in solution at ordinary temper
atures. This has many advantages over eucalyptol whic
was first used as a solvent.

Chloramine T. paste is made by adding chlorazene o
chloramin T. to sodium stearate in hot water, which has bee
neutralized by boric acid.

For sterilizing water Halazone, a by-product in the man
ufacture of saccharin and having a chemical name of sulphor
dichloramino benzoic acid is mixed in the proportion of equa
parts with sodium carbonate, and 8 grams of this mixture i
added to 96 grams of sodium chloride, mixed and pressed
and made into 100 gram tablets. It will keep for 6 to 1
months if preserved in amber bottles. One tablet is added to
liter of water and in 30 to 60 minutes the water is said to b
sterile. It is claimed that in dilutions of 1 in 300,000 wate
will be sterilized in 30 minutes.

Chlorinated lime tablets used for sterilizing water ar
not stable. When 1-20% citric acid is added to the water, i
aids in liberating chlorine, giving the water a pleasant taste
and masks the bad taste in water and also the chlorine
taste. If the citric acid is added to the chlorinated lime tab-
lets, they are of no value after 24 hours. It must be added
to the water after the chlorinated lime has been added.

Formaldehyde is an active antiseptic but its germicidal
powers vary with the organism and conditions. Tubercle
bacilli require 45 minutes in a 5% solution for their destruc-
tion. For disinfecting a tightly closed room sheets may be
wet with the official solution not less than 150 mils for each
1,000 cubic feet and hung about the room and be permitted

o evaporate. The room must be closed for 24 hours, or vaors may be sprayed through the keyhole. Fumigating canles are convenient, and valuable when burned over water. 'ormaldehyde is not very penetrating for mattresses, upholsered furniture, etc., cannot be disinfected. They should be terilized with steam, or burned. The temperature of the oom to be disinfected must not be below 18 degrees C. and here must be not less than 65% moisture in the air for good ffect. The moisture may be added by sprinkling the floor, r boiling water in the room. 150 mils of 40% solution to very 1,000 cubic feet, or of the solid or paraformaldehyde 30 grams may be used. A common formulae is paraformaldehyde 30 grams, potassium permanganate in crystal form 75 grams and water 150 mils. Greater penetration is obtained by using 60 grams of paraformaldehyde. The room is prepared and the potassium permanganate in crystal form over the bottom of a large iron vessel, which is raised from the floor on bricks, the paraformaldehyde is added with water. This mixture is explosive and the reaction is immediate, so care must be observed in leaving the room at once. This mixture is also corrosive, so that at the end of ½ hour, the container should be pulled from the room by an attached cord, care being taken to protect the operator from the fumes. Other mixtures are sodium dichromate 30 grams, a solution of commercial sulphuric acid 45 mils and saturated solution of formaldehyde ½ L. This mixture is destructive to furniture, etc. Chlorinated lime 60 grams, paraform 30 grams are quickly mixed and poured immediately over 90 mils of water, or Chlorinated lime 60 grams, and water 20 mils thoroughly mixed and formalin 75 mils added.

Bulletin No. 42, Public Health and Marine-Hospital Service, states:

Upon the permanent vacation of a room occupied by a person with a contagious disease, whether through death, removal or recovery, the room and preferably the whole house should be disinfected. It recommends the use of 300 mils of formalin and 150 grams of permanganate to every 1,000 cubic feet.

Solid formaldehyde in the form of candles is also very good, if a sufficient number are used, and they are burned over water.

Chlorinated lime 620 grams, formaldehyde 800 mils will equal 250 grams of potassium permanganate and 500 mils formaldehyde and at about 1/6th the cost.

Fire places, ventilators, cracks of doors and windows

and all openings should be closed with cotton, strips of pasted paper, or with the regular rolls of adhesive paper made for this purpose. The exit door must be sealed from the outside. All articles in the room must be well exposed to the action of the gas. All trunks, drawers and boxes should be opened—all articles hung up and exposed to the gas. All cupboards, closets and book case doors should be opened. Several hours after the doors and windows have been open the room is ready for occupancy. Formaldehyde gas is a poor insecticide. It will not injure fabrics, but it is very irritating to the mucous membrane of man. The slower the gas generates, the better the disinfection.

Sulphur is used as a disinfectant by burning 5 lbs. to the 1,000 cubic feet, leaving the room closed for 12 hours; then airing for 12 hours. Sulphur must be burned in an iron vessel standing in water; for sulphur is of no value unless it is converted into sulphur dioxide and this into sulphurous acid. When it comes in contact with moisture, sulphur will tarnish some metal objects.

Copperas is a good deodorant for urinals and is better than sulphur; as the fumes of burning sulphur are very irritating and poisonous, and will corrode metal (this may be avoided by coating the metal likely to be attacked by melted paraffin).

Potassium permanganate in solutions decomposes organic matter, hence it is a good deodorant, but of little value as a disinfectant; as it attacks bacteria more slowly than organic matter. The stains from the permanganate are objectionable.

Mercuric chloride is one of the most powerful disinfectants; but its action is greatly inhibited by organic matter. It also attacks metals. Solutions of 1 to 10,000 usually destroy spores.

Hydrogen peroxide, one of the most widely used and abused germicides, in the presence of pus or other organic matter, is decomposed with a brisk evolution of oxygen. This is of advantage in open wounds as a detergent. If the solution is fresh, its value lies in the evolution of oxygen which is destructive to tissue and may spread the infection; as a styptic it coagulates albumen, and is considered by many as of little use.

The American Medical Journal states that many of the widely advertised antiseptics are wholly useless as disinfectants; that no one should depend on the directions accompanying these preparations, for the prevention of the spread-

ıg of contagious diseases; that the so called chlorides may ave some little value as deodorants but they cannot be used or disinfecting a room and fabrics.

A deodorant is any substance that will destroy foul dors, or the effects of bacterial action, but is not necessarily a disinfectant. Charcoal is a deodorant but not a disinectant. Sulphur is a deodorant; when burned over water, it converted into sulphur dioxide gas, the disinfectant. Formaldehyde is both a deodorant and disinfectant. Bichloride a powerful germicide but not a deodorant.

A germicide is any substance which will kill bacteria.

An antiseptic inhibits the growth and development of acteria without necessarily killing them. Saturated solutions of sugar or salt will preserve meat or vegetable substances from decomposition and decay—these are antiseptic ut not germicides.

The following act as germicides in dilutions of:

Mercuric chloride	1 in 40,000
Potassium cyanide	1 in 500
Iodin	1 in 2,000
Tricresol	1 in 200
Alcohol	1 in 1
Potassio-mercuric iodide	1 in 5,000
Sodium hypochlorite	1 in 1,000
Phenol	1 in 100
Argyrol	1 in 100
Hydrogen peroxide U. S. P.	1 in 50

Boric acid is rapidly absorbed when applied to the skin. t can be found in the urine one hour after application. In rder to be effective, it must be used in saturated solutions. When used for some time as high as 0.2% may be found in he urine. While boric acid is not an active germicide it greatly reduces their virulence. Wet dressings are more effective in Staphylococcus albus and S. citreus than in any other type of infection.

Picric acid in 5% alcoholic solutions is used as a substiute for iodine.

Antiseptic Iodine Solution is composed of Iodine 1, Pottassium iodide 2, Salicylic acid 5, alcohol 70%, 100. This solution is used undiluted for sterilizing the hands at the time of operation. For wet dressings and irrigations, dilutions should be made so that the iodine content is 1 in 1,000. For opthalmia and urethral irrigations a strength of 1 in 25,000 should be used. When using iodine solutions on the skin,

care must be taken to first have the skin dry, or irritation may result.

The bromine-chloroform germicide, composed of 5% solutions of each, is said to penetrate deeper into the folds of the tissues than iodine solutions; and also to have the power of destroying staphylococcus in one minute in comparison with bichloride in 5 minutes. If protected from the light, the solution will keep for some time.

or each essential oil the minimum quantity was determined hich was found necessary to prevent all bacterial growth in dinary neutralized meat broth plentifully sprinkled with ater from a septic source. The inhibiting quantity of henol under the same experimental conditions being 5.6 arts per 1,000, the author obtained the following classification for the essential oils: Thyme 0.7 part per 1,000, marram 1.0, orange peel 1.2, verbena 1.6, cassia 1.7, rose 1.8, ove 2.0, eucalyptus 2.25, mint 2.5, geranium (rose de France) 5, vetiver 2.7, bitter almond 2.8, gaultheria 3.0, geranium (ndian) 3.1, wintergreen 3.2, meadow-sweet 3.3, spike-lavender 3.5, aniseed-tree 3.7, iris 3.8, common cinnamon 4.0, wild hyme 4.0, birch 4.8, anise 4.2, mustard 4.2 rosemary 4.3, umin 4.5, neroli 4.75, lavender 5, balm 5.2, ylang-ylang 5.6, uniper 6.0, sweet fennel 6.5, reseda 6.5, garlic 6.5, lemon 7.0, ajeput 7.2, sassafras 7.5, heliotrope 8.0, cedrat 8.4, turpentine 8.6, parsley 8.8, violet 9.0, camphor 10.0, angelica 10.0, atchouly 15.0. Seven months after inoculation the culture lates were still sterile when the above quantities were used. —[L. Cavel.]

ADVANTAGES AND DISADVANTAGES OF COMMONLY USED DISINFECTANTS

Disinfectant	Efficiency	Adaptability	Destructiveness	Dangerousness
Carbon Monoxide (Harker gas)	Efficient against rats and other red-blooded vermin. Inefficient against insects and bacteria.	Danger from its use intermediate in degree between that of sulphur dioxide and hydrocyanic-acid gas.	Not destructive to materials.	Limited to the destruction of rats or other red-blooded pests, the apparatus must be installed by an expert operator.
Chlorinated Lime	Good, deteriorates rapidly on exposure to air. Keep in sealed containers.	Similar to lime, but with added irritating effects of chlorine, which is slowly liberated.	Bleaches and disintegrates fabrics.	Useful for same purposes lime is used. In addition is most useful for disinfection of drinking water supplies.
Chlorine (liquid)	Excellent for one purpose, i. e., disinfecting water supplies.	The fumes of chorine if accidentally liberated, are dangerously poisonous.	When in solution acts like chlorinated lime solution.	Practically limited to disinfection of water.
Corrosive Sublimate	Very efficient, except in the presence of albuminous matter	Very poisonous if ingested, and irritating to the skin, if used externally in	Corrodes metals. Fixes stains on fabrics.	Limited by its destructiveness and by the presence of large amounts

	matter.	externally in too great concentration or too often.		large amounts of albuminous matter.
Formaldehyde (gas)	Under proper conditions of use a very efficient germicide. It has feeble penetrative powers and its action is rather superficial. Action impaired by cold and dryness.	Not directly poisonous or dangerous to life. Very irritating to eyes and respiratory mucosa.	Very little deleterious effects on materials, danger of fire unless carefully handled.	This is the most generally useful gaseous disinfectant. It does not kill insects and other vermin.
Formaldehyde Solution	Excellent germicide, good deodorant. Deteriorates with age.	Not very poisonous nor likely to be injested very irritating to skin, conjunctiva, and other mucosa.	Very free from injurious effects.	Limited chiefly by its irritating effects. Can not be used as a disinfectant in the sick room. May be used as a fly poison.
Heat	Absolute.	No greater than the every day uses of heat.	Burning absolutely destructive. Boiling injures some	Limited to inanimate things of classes not ex- excluded by its

			fabrics, fixing stains and causes running of colors. It injures cutting instruments. Steaming spoils leather and rubber articles and glued or varnished things.	destructiveness. In the form of hot air in motion it promises a wider field of usefulness.
Hydrocyanic Acid (gas)	The most efficient agent for destroying insects and rats. Has no bactericidal power.	The most dangerous and lethal disinfectant known. Does not give sufficient warning of its presence. Can be used only by experts.	The gas is not destructive to materials, but spilled or spattered acid may cause damage.	Strictly limited to the destruction of insects, rats and other vermin, and to expert application with control over human inmates of the inclosure to be fumigated.
Lime	Good, fresh unslacked lime must be used. Air - slacked specimens are inert.	Poisonous, if ingested in concentrations. Is caustic to the eyes and skin. With ordinary care offers lit-	Quite destructive to many kinds of materials.	Useful only for things which are to be destroyed, chiefly for excreta.

		tle danger.		
Phenol	Highly efficient.	Very poisonous. Destructive to all tissues when concentrated. Benumbs the skin even when diluted.	In the usual dilutions, not destructive of most materials.	Widely useful within the limitations of a liquid disinfectant. Chief objections are its toxicity, effect on the skin, and odor.
Potassium Permanganate ..	Good, in a limited field. Impaired by organic matter.	Slightly toxic if ingested in large quantities.	Stains fabrics.	Very limited, used for hands, as in surgery, disinfecting drinking water containers.
Para formaldehyde	The same as formaldehyde if properly handled. If burned, as in candles, practically inert.	Same as under Formaldehyde (gas).	Same as under Formaldehyde (gas).	Same as above, when properly used.
Sulphur Dioxide	With moisture, a fairly efficient germicide. An efficient destroyer of vermin, with some-	Dangerously poisonous, odor gives warning of its presence.	Bleaches fabrics and ornamental work, attacks metals in the presence of moisture. Some danger of fire.	Now little employed as a germicide. Very useful for destruction of disease - bearing insects and

	what feeble penetrative power.	(P. H. B. No. 42)		vermin. Limited by low penetrative power and destructive action.

THE MICRO-ORGANISMS

Micro-organisms are minute living bodies, either animal or vegetable, visible usually only by the aid of high power microscopes.

The vegetable micro-organisms which we will study belong to the Fungi and the Algae, which are subdivisions of the Thallophytes.

The Algae are plants containing chlorophyll; and the subdivisions, of interest to us, are the Diatomaceae-Diatoms, and the Pleurococcus-green plants on tree barks.

The Fungi contain little or no chlorophyll, and are of necessity parasitic or saprophytic. The fungi are subdivided into the Schizomycetes—fission fungi, and the Eumycetes—usually branching higher fungi, and other subdivisions of little interest to us. The Fungi are subdivided according to the manner of branching and growth.

The Schizomycetes, or Bacteria proper, are unicellular microscopic plants, which can change dead organic matter from complex unstable compounds to simple compounds as seen in fermentation and putrefaction. They are saprophytic or parasitic in their habits. They are found everywhere wherever man, animals and plants live, die and decompose.

Bacteria are the intermediate link between the plant and animal kingdom.

Bacteria are all microscopic in size, rarely not more than 1 micron in length. They are measured by microns, which are about 1-25,000 of an inch and is the unit of microscopical measure. One bacterium weighs about 0.000,000,-0016 milligram.

They multiply on an average of once in 15 to 30 minutes under proper conditions, and the offspring from one bacterium will in 24 hours be over 75,000,000,000,000.

That micro-organisms live for an indefinite length of time has been proven by Galippe, who soaked a piece of papyrus (from the time of Ptolemy) and also ancient Chinese manuscript dating from the 15th century, for several hours in sterile water and found a number of micro-organisms which were viable.

Large colorless plants forming:

1. Delicate threads or mycelia, and classified as to the site of the spores. Phycomycetes.
2. Delicate threads composed of hyphae and mycelia, frequently with colored spores, Molds.
3. Single cell plants, multiplying by budding, Yeasts, or Saccharomycetes.
4. Single cell plants, multiplying by fission or division, or spore formation—Bacteria, Schizomycetes.

The lower forms of Bacteria are classified as to the shape:

1. Spherical bacteria, Coccus.
   a. Dividing in 1 plane so as to form chains, Streptococcus.
   b. Dividing in 1 plane so as to form bunches, Staphylococcus.
   c. Dividing in 2 planes so as not to form chains, Micrococcus.
   d. Dividing in 3 planes so as to form cubes, Sarcine.
2. Rod shaped bacteria.
   a. Without flagella and non-motile, Bacterium.
   b. With flagella and motile, Bacillus.
      1. Bacillus with 1 flagellum, Monotrichic or Pseudomonas.
      2. Bacillus with a tuft of flagella at end, Lophotrichic.
      3. Bacillus with flagella all over body. Peritrichic.
3. Spiral, or shaped like a coiled spring, Spirilla.

The higher forms of bacteria will be studied under Bacteria of the Mouth.

Descriptions of the lower forms of bacteria:

Cocci are round, or oval cells, non-motile, some show Brownian movement.

Streptococci (streptococcus singular) divide by fission in one plane, remaining attached in chain like form by their capsules.

Staphylococci divide in one plane, remaining attached by their capsules, in bunches or clusters like a bunch of grapes.

Diplococci divide in one plane, the two cocci remaining attached in pairs.

Monismopedia divides in two directions in the same plane, remaining attached in groups of four or "tetrads."

Sarcine divides in three planes, or at right angles to the other two cocci, remaining attached in cubical packets of 8.

Bacilli, rod shaped organisms, dividing at right angles

to the long axes, at least three times as long as broad—sometimes reproducing by spore formation.

Spirilla are curved, or comma shaped rods, or spiral filaments reproducing by simple division, or by spores (?)

Spirocheta are curved or coiled like a spring, in long, very thin threads in spiral form.

Spore the resting form.

Flagella the straight or wavy filaments attached to the body of micro-organisms.

Bacteria are usually devoid of chlorophyll, hence are of a necessity:

1. Parasitic.—Those organisms living on a host, living upon or within a living organism and deriving their nourishment from the host.

2. Saprophytic.—Sapron, putrid, and phyton, a plant.—Those which live on dead or putrid organic matter, or prepared food. These are the organisms which cause decomposition, putrefaction, fermentation, et al.

A facultative bacterium is capable of changing its mode of growth as if the skin were irritated, and some of the bacteria of the skin, which are harmless, are rubbed into the skin and boils are produced. If the skin is sufficiently irritated, carbuncles may be produced; or a parasite may have the faculty of living as a saprophyte.

Pathogenic bacteria are those capable of producing disease, as Diphtheria, etc.

Non-pathogenic are those which are incapable under ordinary conditions of producing disease, such as the lactic acid bacteria, which sour milk.

Photogenic bacteria are those which in growing, produce phosphorescence, as Bacillus Phosphorescens.

Phosphorescent are those producing phosphorescent effects in culture media.

Zymogenic bacteria produce fermentation.

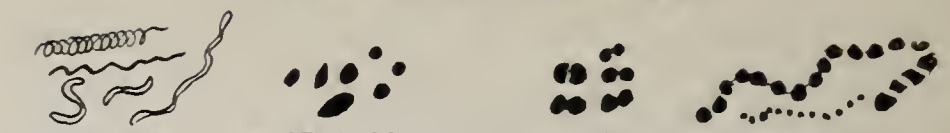
Chromogenic bacteria produce colors at low temperatures and in the dark, as Bacillus Pyocyaneus.

Aerobic bacteria are those which thrive best in oxygen, or in the air.

Anaerobic are those which will grow only without oxygen, or when all the air has been excluded, as the Bacillus tetanus.

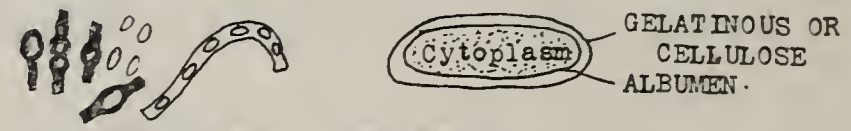
Facultative anaerobes are those which live and grow equally well in oxygen or without oxygen.

SHAPES OF MICRO-ORGANISMS.

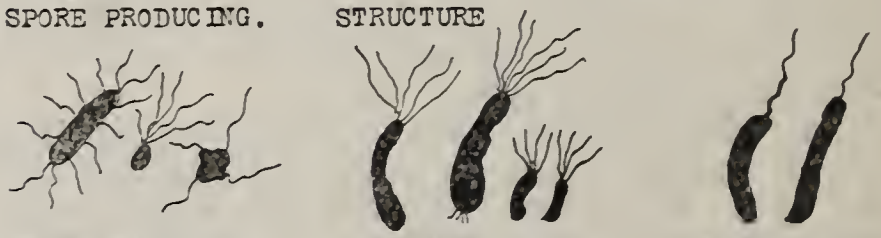


SPIRILLUM. MICROCOCCUS. DIPLOCOCCUS. STREPTOCOCCUS.

STAPHYLOCOCCUS. BACILLUS. BACTERIUM.

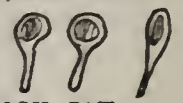


SPORE PRODUCING. STRUCTURE



PERITRICHIC. LOPHOTRICHIC. MONOTRICHIC.

MICRO-ORGANISMS.

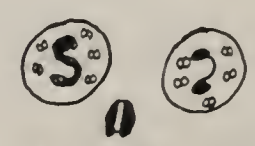


LOCK JAW.

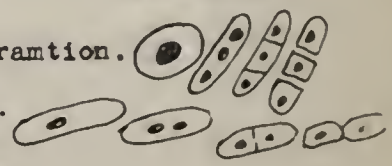
COMPARING THE SIZE.

TYPHOID FEVER. TUBERCULOSIS. CONSUMPTION.

ASIATIC CHOLERA. DIPTHERIA. PNEUMONIA.



GONORRH EAL PUSS.



REPRODUCTION.

Aerogenic bacteria are those which produce gas, as the Coli bacteria.

Luminous bacteria.—Nearly all bacteria when dead and dried, then moistened with water and oxygen added, become luminous. It is destroyed by ether, toluene or by extracting with alcohol.

Reduction bacteria are those which reduce nitrogen as in the root tubercles of the Leguminosae.

Aromatic bacteria produce phenols, hydrocarbons, etc., of which Indol is the most important.

Infection is also divided into:

1. Acute.
2. Chronic, or prolonged.

And also as:

1. Simple infections, where only one kind of bacterium causes the disease, as in Diphtheria.
2. Mixed infections where two or more different kinds of bacteria cause the disease, as in consumption, blood poisons, and influenza and pneumonia.

Infectious diseases are diseases which are produced by invading micro-organisms, as Typhoid fever.

Sporadic infection is where isolated cases of infection occur.

Endemic infections prevail where a disease affects the inhabitants of a given area year after year.

Epidemic infections prevail when a disease appears suddenly and affects a large number of the inhabitants, the number of cases rapidly increasing and decreasing.

Pandemic diseases.—Where an epidemic occurs over a large territory.

Enzootic.—A term used for lower animal diseases which corresponds to endemic.

Epizootic corresponds to epidemic, but is used for lower animal diseases.

Infestation prevails where there are living organisms on the skin, or in the intestinal tract as worms.

Contagious diseases are diseases spread by direct contact, or indirect contact as through toys, letters, clothing, etc.

Contagious zone is the zone of surrounding air, filled with the disease producing micro-organisms, as small pox, or scarlet fever.

Air borne diseases are such as are carried by and through the air, as consumption, scarlet fever, et al.

Water borne diseases are those caused by the organisms

being carried by means of water or milk, as typhoid fever.

There are also Pathogenesis, fevers, congestions, inflammations, itch, uncinerasis, American filaria, Sanguinis homis, Plasmodia malariae, Trypanasomia gambense, et al.

Mechanical and toxic disturbances cause emboli, congested lungs, liver or spleen, necrosis of kidneys or liver, reproduction of scarlet fever, et al.

CAUSES OF SUSCEPTIBLE DISEASES

1. Weakening of surface protection.
2. Inadequate inflammatory reactions.
3. Insufficient supply of receptors.
4. Insufficient activity of antibacterial and antitoxic processes in the body cells and fluids.
5. Virulence of infecting agents.
6. The number of micro-organisms gaining entrance into the body.
7. The mode of entrance, or the site of infection.
   a. Primary, first or original infection, with one kind of micro-organism.
   b. Secondary, or other micro-organisms.
   c. Mixed or concurrent, as colds, where two or more organisms cause the disease.
   d. Terminal, where the body offers little or no resistance and death ensues.

The disease is always caused and governed by the power of the micro-organisms to multiply in the body.

PRIMARY AND SECONDARY CAUSES OF DISEASE

1. Bacteria, as in Typhoid fever.
2. Protozoa, as in Malaria.
3. Parasites, as in the higher animals—intestinal worms, itch, etc.
4. Fungi, as in ringworm, pellagra.
5. Undetermined, as in whooping cough, small pox, etc.

PRIMARY CAUSES (INDICATING)

1. Race.
2. Heredity.
   a. Family—Phylogenitic.
   b. Individual—Ontogenetic.
3. Infancy.
4. Childhood.
5. Old age.
6. Sex.

SECONDARY CAUSES (PREDISPOSING)

A. Environments.
   1. Climate.
   2. Altitude.
   3. Seasons.
   4. Unsuitable food.

5. Unsuitable clothing.
6. Poisons.
7. Occupations.
8. Injuries.

B. Habits.
1. Alcohol.
2. Tobacco.
3. Drugs.
4. Coffee, Tea.
5. Gourmandage.
6. Fast living.
7. Excesses of such kinds as to sap the body vitality.

INFESTIOUS SUBSTANCES, AS TOXINS, AND MICRO-ORGANISMS

Von Behring's Classification:

A. Living pathogenic parasites.
   a. Macroparasites, intestinal forms, pediculi.
      1. Bacteria, fission fungi, each dividing into 2 or more.
      2. Fungi, more complex organisms as Aspergillus, Odia, etc.
      3. Protozoa, as Ameba, Plasmodium, Animalicules.

B. Not living, or alive.

Toxins—poisons are metabolic products of pathogenic bacteria, of unknown chemical composition (many are enzymes) which when injected into a suitable animal are capable of causing an elaboration of specific antibodies.

   a. Animal toxins, as spider or snake venom.
   b. Bacterial toxins, bacterial excretions as seen in Diphtheria.
   c. Intercellular bacterial toxins, those which are not secreted or excreted in soluble form.

Bacterial toxins are of two kinds, according to the bacteria producing them; and are:

1. Exotoxins or Extracellular soluble toxins are the toxins proper, the toxins or poisons which escape from the bacterial cell walls and are soluble in the surrounding media and enter the system by absorption, as in Diphtheria and Tetanus.
2. Endotoxins or Intracellular toxins are those toxins which remain in the bacterial cell and are set free only, when the cell breaks up as in Typhoid and Pneumonia.

These toxins are the cause of various substances to be formed in the blood stream of the patient acting as host to the organism producing them, as:

Antibodies, or immune bodies, substances developed within the blood stream by the toxin. These bodies overcome or neutralize the effects of the toxins and are called and placed with the antitoxins.

Antibodies are formed in the spleen, bone marrow, lymph glands, which are the hematopoietic or blood forming organs.

Antibodies may be subdivided into:

Agglutinins are antibacterial bodies in the blood, which cause the corresponding bacteria to become clumped or agglutinated, as in the agglutination test for typhoid fever (see pages on typhoid fever).

Precipitins are substances formed artificially by repeated

injections of bacteria, or foreign blood into rabbits and other animals. They will produce a blood serum which will precipitate the corresponding blood or bacteria, as in testing blood stains for human blood or in the pneumococcus precipitation tests (see pages on).

Cytolysins are formed when suspensions of cells are injected into animals, producing certain substances which are injurious to similar cells to those injected, forming destructive bodies for that particular kind of cells only.

Opsonins are substances formed in the blood which act upon or prepare the bacteria for engulfing by the leukocytes.

Bacteriotropins are substances similar to opsonins.

Antigens are substances formed in the blood which will cause the formation or appearance of the antibodies. Various kinds of antibodies may be produced by the same antigen.

Complement or alexin is a non-specific substance found in fresh blood serum of practically all animals. It is not increased during but takes part in immunization.

Amboceptors are immune bodies which are insufficient without the complements to produce immunity, and probably act by uniting the complement to the antigen; the bacteriolysins and cyto-toxins are antibodies of this order.

Allergy means to alter the condition of an animal into whose tissues an antigen has been injected.

A specific body is one having a special affinity for the antigen, calling it forth, or causing it to be made, as in Diphtheria antitoxin, the antibody will neutralize diphtheria toxin only.

BACTERIAL VACCINES OR BACTERINS

Bacterial vaccines were discovered by Wright. They are suspensions of killed bacteria in physiological salt solution. They are prepared by growing the specific organisms in suitable culture media, killing the bacteria, counting and suspending in salt solution, preserving by adding tricresol, labeling and sealing the ampule containers.

Bacterial vaccines may cause agglutinins, opsonins and bacteriolysins to form at the same time, and work together as one or each may work separately.

Bacteriolytic power brings about the digestion of certain specific bacteria.

Specific organisms are those bacteria which cause the disease.

When the body is invaded by a specific pathogenic microorganism, certain non-specific antibodies are called forth. These antibodies in the blood stream oppose all pathogenic invasions. The body produces a specific antibody which is directed against that particular invading organism.

The body always has the non-specific antibodies, but usually not sufficient. Therefore a bacterial vaccine must be introduced for the specific invading organism, and this may be of two kinds:

1. Heterogenous, that is the vaccine is made from organisms obtained from stock, or laboratory cultures.
2. Autogenous, or personal vaccine, where the vaccine is made from the organisms obtained from the patient suffering from the disease.

In both kinds of bacterial vaccines, the same object is desired, that is, to cause the body to form antibodies, differing from antitoxins in that the antitoxins contain the antibodies, while the bacterial vaccines cause the body to make its own antibodies.

In obtaining the organism for making bacterial vaccines, all asepsis must be observed. In all cases no antiseptics must be used on the surface from which the culture is to be obtained.

Collecting the specimen for culture.

Urine—30 mils are sufficient, collected in a sterile bottle—first sterilizing in men, the glans penis and meatis by washing with soap and water—then with 50% alcohol, rejecting the first portion of urine voided. Women should be catheterized. 24 hour urine specimens are not essential, except in special cases. The sample should be taken 3 hours

after the mid-day meal, or the morning urine is perhaps best.

In rheumatic conditions the joints should be massaged before passing the urine. Bacterial cultures from urine are easily obtained, and more easy to culture than those from blood. The specimens must be kept cool and cultured as soon as possible.

Feces must be obtained urine free and specimens from the first and last portions are to be cultured. Make several smears or films, examine and make cultures.

Sputum.—The night before obtaining specimen, the mouth is to be cleansed with sterile water, and teeth thoroughly cleansed. In the morning cleanse the mouth and teeth thoroughly. Gargle with sterile water, rinse mouth thoroughly. The sputum is expelled by a pulmonary coughing act into a wide mouth sterile bottle with a sterile cork, and tightly corked. The outside of the bottle must be washed off with alcohol or phenol solution. Remembering that saliva alone will not give results and that no disinfectants or antiseptics are to be added to the bottle or contents. Make several smears and examine; culture as directed.

Pus from boils, acne, pustules, infection, et al.—Cleanse part with soap and water; if it is desired to puncture abscesses, cleanse with soap and water, followed by alcohol or ether, before puncturing, and take specimens with capillary tube or diphtheria swabs. Smear several slides, stain and examine. Culture.

Pulmonary abscesses.—Sterilize the chest wall as directed for blood cultures, using a 5 mil all glass sterile syringe, containing 3 mils of sterile peptone broth. Inject, aspirate and tube for culture.

Blood may be taken as directed under the Widal test, or by sterilizing the skin of the elbow with soap and water, followed by alcohol, ether or iodine, before puncturing. Take specimen with capillary tube or all glass syringe, puncture the vein and draw the blood. Or the joint of the middle finger is sterilized. If the hand is cold it may be immersed in hot water; compress and squeeze so as to drive the blood towards the finger end. Prick deeply with a broad lance across the lines of the skin and collect the blood into a small test tube or capillary tube. It is best when taking the blood for culture to distribute directly into sterile flasks containing 500 mils of sterile broth. Smears or films of blood should be made, stained and studied. If the malaria parasite is desired the blood should be taken, just after a chill. It is

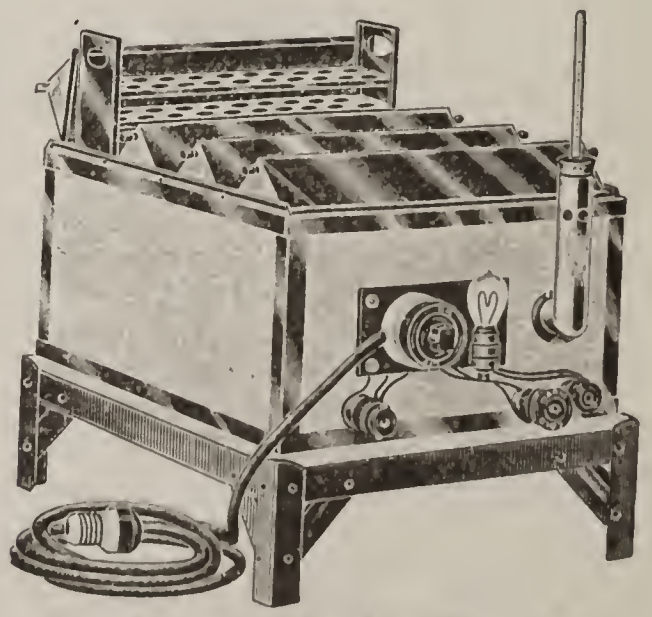
sometimes advisable, especially in nervous patients and children, to take the blood from the toe.

Cultures from gastric contents. A test meal as the Ewald breakfast consisting of 2 slices of dry toast and 2 cups of weak tea, or better water. After one hour, the meal is returned. The stomach must be emptied before giving the test meal, which should be given the first thing in the morning. Examine the smears and cultivate the contents.

Selecting the micro-organism.

Note the organisms present and determine the causative organism or organisms.

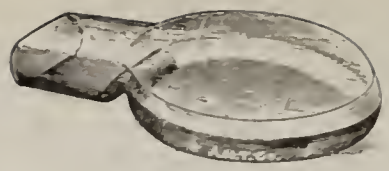
Cultivate 24 hours by inoculating 2 sterile agar slants, 2 Loeffler's blood serum and other media if necessary. After having obtained a pure culture of the offending organism, place 5 mils of sterile normal salt solution in a cotton plugged sterile test tube; place the test tubes in water bath at 60 degrees C. Make a thick emulsion by rubbing gently the growth with a heavy platinum needle and mixing with the normal salt solution until a uniform emulsion is obtained.— Mix with the remaining portion of the sterile salt solution. Seal the top of the test tube in the flame. After cooling, shake the emulsion for 15 minutes. Cut off the top of the sealed tube with a file. Withdraw by means of a sterile pipette, a small quantity of the emulsion and deposit in a watch glass. Then place the pipette in a solution of tricresol.

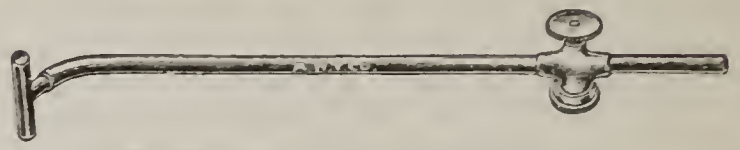


Electric Water Bath

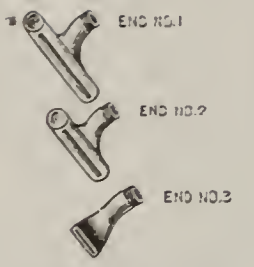
Cover the watch glass with another watch glass to prevent evaporation, then reseal the tube of emulsion. Wrap it in sheet lead and sink in the water bath for one hour. Obtain a count of the organisms as directed under the Opsonic index (See pages on Blood). Use the ruled eyepiece, or a good substitute may be made by unscrewing the upper eyepiece lens and cutting a piece of cardboard to fit inside. Then cut a square hole in the center of the cardboard, and cement with canada balsam 2 hairs at right angles across this hole. Use your own blood and count 500 erythrocyte cells (instead of leukocytes as in the opsonic index). Obtain the number of erythrocytes and the number of bacteria present. Then the number of erythrocytes present are to the number of bacteria present as five million are to X. Decide the dose per mil, and the amount of the emulsion to be placed in each ampule. After one hour has elapsed, remove the emulsion from the water bath; file top off the test tube; and withdraw with a sterile pipette a small amount of the emulsion; and place in a test tube with melted dextrose agar (held at 45 degrees C.) Make a shake culture and incubate. This is a test for viability of the supposed killed organisms. If the emulsion is too thick or heavy, dilute with the salt solution and withdraw a sample, and culture as above directed. Place 0.25% tricresol in the emulsion; shake well; and withdraw by a pipette the required amount; and place into ampules, seal the ampules and label with the name of the organism; the number of organisms per mil and the date. Instead of scraping the organisms from the culture a very good apparatus, which is illustrated, facilitates the work.

25684. Culture Flask,

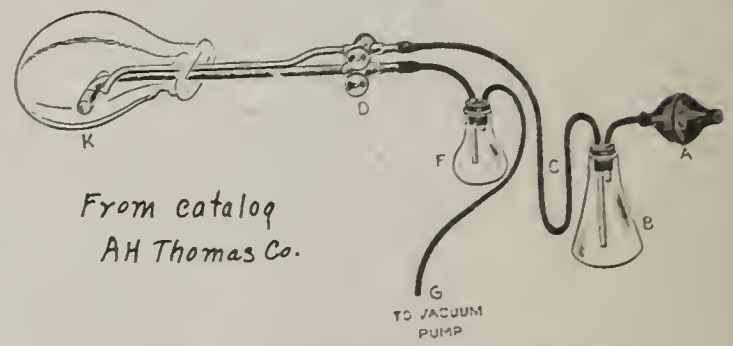




No. 24696. Vacuum Collector for Bacteria, simple form.



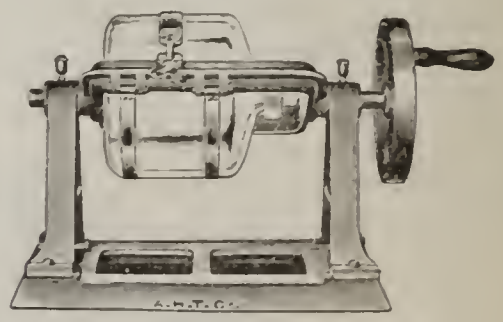
Three styles of End Pieces as furnished with Vacuum Bacteria Collectors, Nos. 24694 and 24696.



From catalog
AH Thomas Co.

No. 24695 Vacuum Collector for Bacteria, complete outfit.

Bacteria Grinding Jar,



Bacteria Grinder, Single Jar,

DOSAGE OF BACTERINS

The size of a dose of a given bacterin, like that of a drug, depends upon a number of factors, such as age, sex, body-weight, idiosyncrasy, and so on. But in general, the adult dosage of the bacterins in common use is as follows:

Staphylococcus pyogenes albus..100 millions to 1,000 millions
Staphylococcus pyogenes aureus, 100 millions to 1,000 millions
Streptococcus pyogenes.....30 millions to 100 millions
Streptococcus erysipelatis.....50 millions (daily)

Streptococcus viridans and S. rheumaticus.....50 millions to 500 millions
Pneumococcus.....20 millions to 100 millions
Gonococcus.....100 millions to 1,000 millions
Micrococcus catarrhalis.....25 millions to 10 millions
Bacillus coli communis (color-bacillus).....20 millions to 200 millions
Bacillus typhosus (typhoid-bacillus) (immunizing).....500 millions to 1,000 millions
Bacillus typhosus (typhoid-bacillus) (therapeutic).....100 millions to 500 millions
Bacillus influenzae.....50 millions to 200 millions
Bacillus of acne.....10 millions to 100 millions
Bacillus of Friedlander.....75 millions to 300 millions
Meningococcus (immunizing).....500 millions to 1,000 millions
Bacillus tuberculosis (non-virulent).....100 millions to 1,000 millions

Pneumococcus antigen is a bacterin which has been partly autolyzed pneumococci. This process removes a large amount of the toxic properties.

LIPOVACCINES

Lipovaccines are made by growing the bacteria, drying them in vacuo, or freezing, when they have the appearance of flaky masses, grinding them for 24 hours with steel balls; then mixing with 90% sterile lanolin at 90 degrees C. for 10 hours on a water bath, or in the autoclave for 15 minutes Bacillus pertussis (Bordet's bacillus), 50 millions to 500 millions at 15 lbs, pressure, or by mixing or grinding with olive oil 10 days or with sweet oil for 3 days. They are injected subcutaneously and are said to eliminate the objectionable features caused by the ordinary bacterial vaccines.

SEROBACTERINS

Sensitized vaccines are made by growing the bacteria in immune serum which is usually obtained from the goat, heated to 56 degrees C. for ½ hour, normal salt solution added and again heated from 6 to 12 hours. Then centrifuged and washed 3 times, in normal salt solution. A suspension in normal salt solution is made, the suspension then heated for 1 hour at 60 degrees C., tested for sterility, counted and placed in ampules. It is said to produce less local reaction and to permit larger doses, and to produce immunity in 24 hours. However sensitization may take place and cause severe reactions.

Detoxicated vaccines are made by dissolving the bacteria in alkali and precipitating with acid, repeating this process until the supernatant liquid gives no precipitate with picric acid. A modificated detoxicated vaccine is made by washing the first precipitate with a solution of sodium phosphate or weak acid to remove the toxins. The precipitate obtained by either process is dissolved in alkali or mixed with a solution of sodium phosphate for use.

SERUMS

Serums are the clear liquid which separates from the blood corpuscles and other constituents, entering into the formation of the blood clot, during the process of coagulation.

Antitoxic serums are the clear liquid separated from the blood obtained from an animal immunized to that certain disease. They are prepared by first producing the toxin of the specific bacteria by growing the bacteria, and filtering off the toxin; and injecting the toxin into the animal, or by injecting killed bacteria into the animal. A very small amount is injected at first, causing a reaction, after this reaction has subsided, a larger dose is given and this repeated until no reaction takes place, and the animal is said to be immune. The animal is bled, the serum separated from the clot, tested for sterility and standardized. This is then placed into containers and labeled stating the number of units it contains. (See pages on Antitoxins).

The antitoxin unit for antidiphtheric serum is: That amount of antitoxin which will protect a 250 gram guinea pig against 100 times the L. dose (The L. or lethal dose means the smallest amount of toxin which will kill the guinea pig in the presence of 1 unit of antitoxin). This amount is the unit.

The antitetanic unit is the smallest amount of antitoxin which will protect a 350 gram guinea pig against 1,000 times the L. dose.

Anaphylaxis occurs in about 10% of persons on whom bacterial vaccines or antitoxins are used, and it is—the lack of resistance—it is also called serum sickness. Anaphylaxis is the opposite to prophylaxis. It often occurs after the second injection. The first injection causes a sensitization—a state of hyper-sensitiveness and affects the ennui said to be due to a foreign protein and not to the antitoxin, for many persons exhibit this anaphylaxis, as in strawberry rash, which appears after eating strawberries, or the rash which appears on some persons after eating eggs.

When proteins are injected into the body, as in the antitoxins, the antibodies which are formed split up and eliminate the foreign proteins. Toxic bodies are also formed and released. Anaphylaxis is manifested, immediately to about 12 hours after injections by skin eruptions, malaise, joint pains, et al., which rapidly pass away and leave no permanent symptoms. This is often avoided by repeated injections of antitoxin or bacterial vaccines. No special treatment is re-

quired for the skin eruptions; soothing lotions, cathartics and sedatives may be used. In severe cases, shock may appear and be followed by collapse.

The treated proteins split into 2 or more parts, one of which is poisonous and the other non-poisonous. The non-poisonous are the ones used in immunization.

TOXINS, ANTITOXINS, ANTIBACTERIAL SERUMS AND BACTERIAL VACCINES

ANTITOXINS OR ANTITOXIC SERUMS—Only neutralizing bacterial toxins.

Serum Antidiphthericum, U. S. P.

Serum Antidiphthericum purificatum, U. S. P.

Serum Antidiphthericum Siccum, U. S. P.

Serum Antidiphthericum Siccum, U. S. P. The potency is not less than 4,000 units per gram, and the dose is Hypodermic 10,000 units and Protective, 1000 units.

Serum Antitetanicum purificatum, U. S. P.

Serum Antitetanicum Siccum, U. S. P.

The potency is not less than 100 units per mil, and the dose is Hypodermic 10,000 units and Protective 1,500 units.

ANTIBACTERIAL SERUMS OR BACTERICIDAL SERUMS—Prevent bacterial development.

Antipneumococci Serums.

Antiplague Serums or Yersin's Serum.

Antistreptococci Serums.

Antituberculosis Serums—Antituberculins (see page on Tuberculosis.)

Scarlet Fever Serums or Marpman's Serum.

BACTERINS OR BACTERIAL VACCINES—Cause the body to form antibodies, and are suspensions of killed bacteria in normal salt solution.

Homologous, or personal, or autogenous, bacteria cultured from the patient.

Heterogenous, or stock bacterins, from other sources than the patient.

Mixed, containing more than one kind of bacteria.

Bubonic plague bacillus—Haffkin's plague Bacterin.

Coli bacillus Bacterin.

Neoformans bacillus Bacterin.

Pyocyaneus bacillus Bacterin.

THE SOURCE OF THE TUBERCULINS

Cultures of Bacillus Tuberculosis, in 5% glycerin bouillon

A. Tuberculin old, Koch's old or T. O., contains both the soli and soluble portions of the culture.

a. 6 weeks culture, evaporated to 1-10 its volume, filtere and mixed with 50% glycerin.

From this diagnostic reagents are made; these are:

1. Tuberculin Ointment, used in the Moro skin test.
2. Tuberculin purified used in the Calmutte eye test.
3. Tuberculin used in the Von Pirquet injection test.
4. Tuberculin Suppositories, used as curative.

Filtering tuberculin old, the filtrate is called:

B. Tuberculin or B. F. (Bouillon filtrate) or Denny's. Thi is used as a curative and contains the soluble products.

C. The residue or Tuberculin Residue or T. R. from whic are made:

a. Tuberculin T. R. Concentrated.
b. Tuberculin T. R. Dilute.
c. Tuberculin B. E. or Brazilian Emulsion, which consist of the organisms dried, powdered and mixed with 50% glycerin; 1 mil equals 1 mgm. of the solid.
d. New Tuberculin. The organisms dried in vacuo, pow dered, washed in salt solution, centrifuged, ground an 20% glycerin added. 1 mil equals 2 mgms, of the solid

Antibacterial serums are made by injecting a male shee with a killed 24 hour culture of the organism, using about 1 square inches of surface culture; in 7 days a second injectio of the killed organisms, using 30 square inches as the dose And the third week an inoculation consisting of 18 squar inches of the unheated culture is given. The first and secon doses are of cultures heated ½ hour at 65 degrees C. Th fourth inoculation consists of a dose of 36 to 45 square inche of the culture. Different strains of organisms give differen results; it is best to make the serum by using organism taken from the patient on whom the serum is intended to b used.

In making antistreptococcus serum, the horse is used. I is inoculated every other day for about 2 months, then 4 t 6 liters of blood are taken. The dose for man is 40 to 8 mils and repeated in 4 hours if necessary, or 20 to 40 mil may be injected every 6 to 8 hours. The strength is undc termined as no method has been devised for determining it.

Antistreptococcus and gonococcus serums as yet are o little value. These organisms do not form soluble toxins bu seem to cause the diseases by breaking up into ultra micro

scopic particles which cause irritation to the cell and its protoplasm.

Antityphoid Bacterin, or Typhoid Bacterial Vaccine.

Mixed Typhoid Bacterin (containing coli and other bacteria).

Streptococcus Bacterin.

Staphylococcus Bacterin.

Mixed Staphylococcus Bacterin (S. alba, S. aurens, S. citreus).

Antimeningitic Serums.

Antidysenteric Serums.

Anthrax Serums.

And many others.

TOXINS AND ATTENUATED FILTERABLE VIRUS VACCINES

Antivenine, or snake vaccines.

Cancer vaccines, or Gilman's vaccine, injections of dried cancerous tissue.

Erysipelas vaccine.

Prodigiosus Toxin, used for cancer and other malignant growths.

Rabies vaccine (see page on Rabies).

Virus Vaccinicum—Small pox vaccine—In capillary tubes only.

IMMUNITY

That which prevents the gaining of a foothold by the disease organisms in the animal body, or that which neutralizes their harmful products or destroys the parasites, or the power living organisms possess to resist and overcome infections. Or as defined by Styles—The power of resistance of the human body and the aggressiveness or virulence of the invading organisms. It is the resistance.

Immunity is only relative, the fowl that is immune to tetanus, is made susceptible by lowering its vitality, by suddenly plunging it into cold water.

Acquired immunity is when the patient has recovered from the disease or has been given immunity from inoculations—as in Small pox and Diphtheria.

Natural immunity is when the patient has had the disease or infection by the organism and its products, and there are developed within the body certain specific antagonistic properties to that certain organism which is usually demonstrated in the blood stream or other body fluids, and to these bodies the term agglutinins is applied; or it is that which causes evenly distributed organisms to come together and form clumps—as Widal reaction. Opsonic power is that which so alters the resistance of bacterial organisms that they may be engulfed by the phagocytes—the name applied to leukocytes after they have engulfed bacteria.

The word immunity comes from the Latin language and means exempt.

1. Natural immunity. This is found in lower animals only—as seen in measles, mumps, whooping cough, typhoid fever, etc.—diseases to which man is susceptible, but which lower animals do not acquire, so that the lower animals are said to be immune to these diseases. It is usually inherited, as seen in the Negro, who is immune to the yellow fever; or in a mare which has recovered from tetanus, and whose colt is immuned to tetanus.

Natural immunity is rarely absolute, such as the chicken which is immune to anthrax but after a sudden chill readily takes the disease. Man after recovering from pneumonia is somewhat immuned, but is very susceptible after a chill or cold.

2. Acquired immunity is that immunity which results after recovery from an attack of pathogenic micro-organisms, such as small pox, measles, scarlet fever, etc., but rarely acquiring the disease the second time. There is no immunity to colds or grippe, unless by that obtained from the use of

bacterial vaccines. This form of immunity is due to the antibodies.

Immunity may be classed as:

1. Active—that immunity acquired after having the disease, or that immunity produced by the patient.

2. Passive—that immunity obtained by the use of antitoxins, etc.

HISTORY OF IMMUNITY

The first practice of immunization was by the Psylli tribe f Indians, who acquired a certain form of immunity against nakes by drinking water which had been infested by the nakes. The Mosambique tribe of Indians used dried liver nto which the snakes had bitten; this was used very much he same as we use small pox vaccine today. Mithridates fed oison to ducks and used the duck blood as a protective. enner used cow pox vaccine. The ancients believed that iving acids would produce alkaline—empiricism to ration- lism.

Hogs are prone to hog cholera; horses and mules to in- luenza; sheep and heifers to black leg, et al. All may have onsumption, anthrax, glanders, tetanus, actinomyces, cow ox, etc. The goat, dog and hog rarely have consump- ion. Algerian sheep and rats are immuned to anthrax, also irds, pigeons and some rodents. Snake venom is poisonous o all animals but the hog.

Vaccination was known to the ancients before Christ; Turkey and Mexico practiced vaccination against small pox y using the virus from the pustules. But vaccination was ot placed on a practical basis until 1799, when Jenner dis- overed the cow pox vaccine and gave it to the public in 1798. Vaccination was used in Germany in 1800 and has been com- ulsory ever since; and Germany has been practically free rom small pox since that time. In 1840 vaccination against small pox was prohibited in England by the parliament and has not been enforced since; hence the reason small pox has been so prevalent in England.

Pasteur established the principle of protective inocula- ions and attenuations lessening the virulence.

Tousant attenuated sheep blood by heating at 55 de- grees C., making the toxin less virulent.

Pasteur improved this attenuation by avoiding sporula- ion by heating to 42 degrees C., which was much better.

Koch established microbe specificity of infectious dis- eases, and attenuated by heat, sunlight and dessication as in tuberculins (see page on tuberculins).

ARTIFICIAL IMMUNITY

1. This is produced by injecting bacteria or their pro- ducts, and as a result antibodies are formed which neutralize the toxin and destroy the specific bacteria. These antibodies are supposed to be thrown off from the receptors. (See Ehr-

lich's theory), or may remain attached to the cell, and remain potent for an indefinite time and so confer a more or less lasting immunity.

2. It may be produced by the serum of animal or man immunized actively, which is injected into a second animal thus conferring on the second animal an immunity; but as the cells are only passive and take no active part in the production of this immunity, this is passive immunity. If this serum which is introduced in passive immunity, only neutralizes the toxic products of the specific bacteria, it is called antitoxic serum, but if it destroys the organisms it is called antibacterial serum, or antimicrobic serum; and the immunity is termed antimicrobic passive immunity. Some serums are both. Some bacteria possess a toxin which is given off when the organism is living and others only when the organism is dead and broken up. Diphtheria and tetanus are antitoxic, nearly all others are those which throw off the toxin from the cells in response to the injury incident to the attack of the cell. The others are microbic. These protoplasmic particles thrown off from the cell enter the circulation and represent the entire antitoxic immunity. They are capable of uniting with the molecule (Toxin) and neutralizing its toxic powers, binding the combining end (Haptophore group); that is, the toxin incapable of attaching itself to a cell, so that the poisonous end of the toxin (toxiphore group) cannot have access to the cell. Two factors must be considered:

1. There is a protoplasmic particle, similar to the antitoxin molecule which in itself has no power of injuring its specific bacteria. It is the specific product of a specific bacterium or a foreign cell against the body cell attacked—withstanding the temperature of 55 degrees C. or more, and of itself incapable of injuring the bacteria, in response to whose attack it was produced.

2. The factor in bacteriolysis of the specific bacteria, or the hemolysis of the specific foreign bacteria is sometimes present in the serum of every animal and is capable of disintegrating a foreign cell or bacteria, through an intermediary amboceptor (amboceptor and intermediary body are the same) and sometimes called complement. The complement cannot act or destroy invading bacteria or cells unless the amboceptor is present to make the necessary connections. The complement is destroyed at 55 degrees C., so that if the serum of an immune animal is heated this high, the comple-

ment is destroyed. The amboceptor it contains which is not injured by this temperature, is capable of destroying cells or bacteria, unless the complement which has been replaced by a fresh complement, is destroyed.

This is done experimentally by adding the serum of a non-immunized animal, which contains the complement, but no specific immune body (Amboceptor) to the heated serum —this is termed activation or activated serum. When immune serum is heated to 56 degrees C., it is said to be inactivated. When a mixture of bacteria and cells remain in contact with their specific immune serum, which has been inactivated, the amboceptor attaches itself to the bacteria or cells; so that on adding serum with the complement, these bacteria or cells are prepared so that the complement can disintegrate them—this is termed sensitizing and the treated cells are said to be sensitized.

Serums may be of two kinds:

1. Normal serum, obtained from normal healthy animals.
2. Immune serum, obtained from animals immunized to certain bacteria.
   a. Antitoxic.
   b. Antibacterial.

Immune serums are usually obtained from the horse on account of the horse serum being non-poisonous to man (Cow serum is very poisonous) and the horse usually is a healthy animal. Large quantities of serum are available and horses stand the toxin injections. Normal sheep serum may be used instead of normal horse serum when anaphylaxis from horse serum is expected.

IMMUNIZING AGENTS

A. Active.
   1. Natural.
      a. Normal or inherited.
         Bacteriolysins—kill and dissolve bacteria.
         Opsonins—prepare bacteria for ingestion by the leukocytes.
         Phagocytosis—the engulfing of bacteria by the leukocytes.
      b. Augmented—immunizing in infectious diseases.
   2. Artificial.
      a. Modified toxins and antitoxins.
         Toxins.

Living viruses—small pox vaccine, rabies vaccine.
Precipitins—precipitate bacteria.
Agglutinins—cause clumping and render bacteria inactive.
Lysins—dissolve bacteria.
Antigens—colloidal protein molecules, act against foreign proteins.
Antibodies—include opsonins, antitoxin molecules, antigens, bacteriocidins, bacteriolysins and agglutinins.
Haemolysis.

b. Bacterial.
Bacterial vaccines.
Stock.
Autogenous—personal.
Bacterial sera.

c. Chemotherapy—Salvarsan.

B. Passive.

1. Antitoxins—as antidiphtheric sera—neutralize toxins produced by bacteria.
2. Antibacterial sera.
3. Drugs—nuclein, lobelin, phosphorus, etc.
4. Prophylactics—against colds, typhoid fever, etc.
5. Phytotoxins—plant poisons—ricin, abrin, etc.
6. Zootoxins—phrynolysin (toad venom), arachnolysin (spider venom) crotalin (rattlesnake) scorpion, bee and other animal poisons.

Inherited.

a. Racial or Phylogenic—as observed in different orders, families, genera and species of animals.
b. Individual or Ontogenic—as observed in different individuals of the same species.

Induced.

A. Active.

1. Natural—due to normally induced infections with diseases which produce immunity to subsequent attacks, such as diseases of childhood and acclimation to others.
2. Artificial—due to the uses of modified toxins, bacterins and direct inoculation with the specific bacteria.

b. Passive—the use of antitoxins and other disease preventatives.

Injecting antitoxins furnishes the body with receptors. Injecting killed bacteria stimulates the body to make its own receptors.

Hektoen's Classification

Products of Immunization.	Antigens.
Antitoxins.	Toxins.
Antiferments.	Ferments.
Precipitins.	Precipitinogens.
Agglutinins.	Agglutinogens.
Opsonins.	Opsonogens.
Amboceptors or lysins.	Lysogens.
Antiantitoxins.	Antitoxins.
Antiagglutinins.	Agglutinins.
Anticomplements.	Complements.
Antiopsonins.	Opsonins.
Antiamboceptors.	Amboceptors.
Antiprecipitins.	Precipitins.

VARIOUS THEORIES OF IMMUNITY

1. Ehrlich's Side Chain Theory consists of: The action of toxins on the cells is chemical. The toxin unites with the cell on account of some chemical group in the molecule toxin which has a chemical affinity for some specific group in the cell protoplasm. This group of the toxin combines with the cell and is the haptophore, while the group in the protoplasm combining with the toxin is the receptor.

The toxophore groups are those where the toxin loses its poisonous power, but still retains its combining power. If all receptors are combined with the toxic molecules in this last group, the cell is not injured by the corresponding active toxin.

2. The Gross ferment theory. Where the organism destroys the cells, the liberated protein causes antibodies to form, thus causing bacterial ferments which are active even after the destruction of the organisms. The ferments may be active or inactive; the active group is neutralized by the inactive, resulting in a zymogenic condition, becoming active only on dissociation. The antibody is called immune. The immunity reactions are composed of union of the antigen with the immune body, (toxin and antitoxin bindings), or a like process plus absorption of the ferments of animal organisms.

3. Haughan and Abderhalden protein split poison theory. All proteins have a nucleus, which is poisonous, producing the same lesion in animals, regardless of the protein source. Specific enzymes are developed upon the injection of a second dose of the same protein. These enzymes disintegrate

it—some of the cleavage products being toxic. The anaphylactic intoxication often follows.

4. Pick's Specificity Theory. The different species have distinct differences in their protein chemistry.—Two kinds of specificity in each protein molecule. One thermolabile—easily altered by physical measures such as heat or cold and not changing the protein chemical nature, but changing its antigenic properties. The other thermostable—only marked chemical alterations of the antigen modify it, hence the proteins act as foreign proteins and retain their chemical activities.

5. Physical chemistry theory. Antigens are colloidal and are divided into two groups. The first adsorption, binding in a collateral system, without changing its degree of dispersion. The second—alterations, a binding that effects a thorough change.

THEORIES OF THE CAUSES OF IMMUNITY

1. Exhaustion—Pasteur, that the organism develops only as long as food lasts and only in suitable media.

2. Unfavorable culture body—Baumgarten, the organisms develop only in favorable media, as some must have oxygen, others blood serum, etc.

3. Noxious retentions—Chauvay, dead bacteria may be retained in the body for years.

4. Phagocytosis—Metchnikoff, the engulfing of bacteria by the leukocytes.

5. Bacterial power of blood serum. Normal serum is antibacterial before the patient has the disease, but more so after recovery.

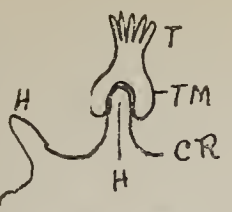
6. Increase of natural powers, such as stimulating the normal powers by good food, et al.

7. Antitoxic serum destroys poisons, the separation of bacteria and toxin by injections of antibodies.

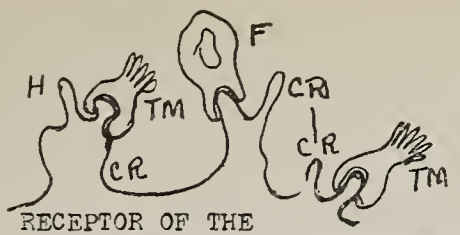
8. Chemotaxis—the attraction of leukocytes and bacteria. Two phases—positive and negative as demonstrated by the opsonic index.

9. Temperature, factors favoring chemical changes—kills bacteria.

10. Poultices, aid and give relief from congestions, by causing an exudation of plasma, and increase and stimulate the leukocytes.



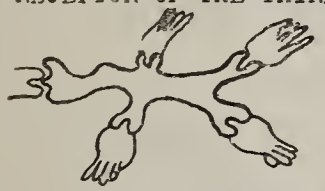
RECEPTOR OF THE FIRST ORDER.



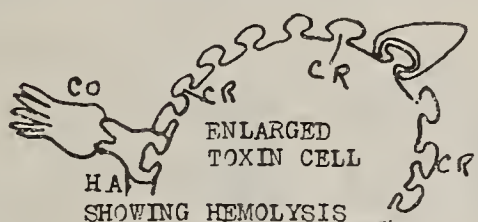
RECEPTOR OF THE SECOND ORDER.(WHERE SOME FOOD SUBSTANCE UNITES WITH THE CELL RECEPTOR)

RECEPTOR OF THE THIRD ORDER.

COMPLEMENT (ZYMOTIC GROUPE OF COMPLEMENTS)

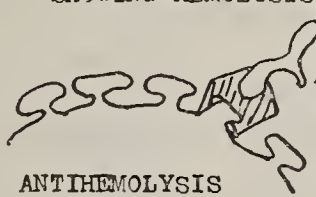


AMBOCEPTER WITH MORE THAN ONE COMPLEMENT



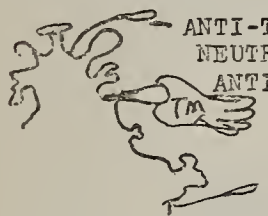
ENLARGED TOXIN CELL SHOWING HEMOLYSIS

ANTICOMPLEMENT UNION WITH COMPLEMENT.



ANTI-COMPLEMENT

ANTIHEMOLYSIS



ANTI-TOXIN MOLECULE NEUTRALIXING TOXIN BY ANTI-TOXIN MOLECULE.

ANTITOXIN PRODUCTION.

HAPTOPHORE

T.TOXIPHORE,TM.TOXIN MOLECULE H.HAPTOPHORE,CR.CELL RECEPTOR, CO. COMPLEMENT, F. FOOD SUBSTANCE OR PRODUCT OF BACTERIAL DISINTEGRATION,HA.HEMOLYSIS.

EHRLICH'S SIDE CHAIN THEORY OF IMMUNITY.

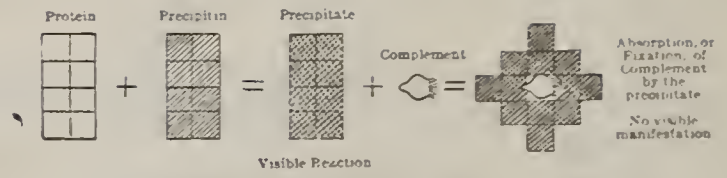
ENDOTOXIN ANTIBODIES PRODUCED BY THE BACILLUS

SENSATIZED BACILLUS TOXIN COMBINED WITH COMPLEMENT

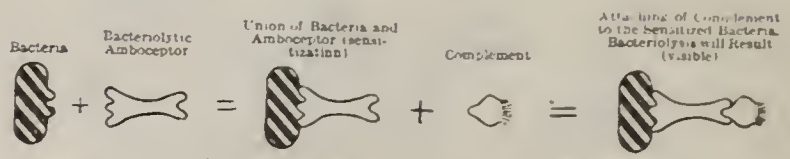
LIBERATED ENDOTOXIN.

ANTI-BODY(ANTI-ENDOTOXIN STIMULATED BY LIBERATED ENDOTOXIN)

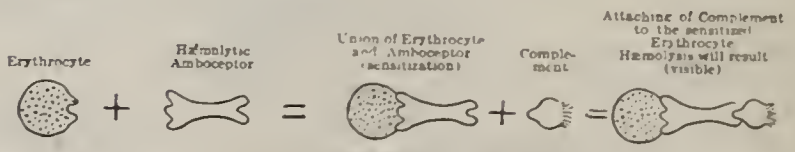
THEORY OF TYPHOID IMMUNIZATION



FIXATION OF COMPLEMENT BY PRECIPITIN REACTION


Bacteria
Bacteriolytic Amboceptor
Union of Bacteria and Amboceptor (sensitization)
Complement
Attaching of Complement to the Sensitized Bacteria. Bacteriolysis will Result (visible)

FIXATION OF COMPLEMENT BY BACTERIOLYSIS


Erythrocyte
Hæmolytic Amboceptor
Union of Erythrocyte and Amboceptor (sensitization)
Complement
Attaching of Complement to the sensitized Erythrocyte Hæmolysis will result (visible)

FIXATION OF COMPLEMENT BY HÆMOLYSIS

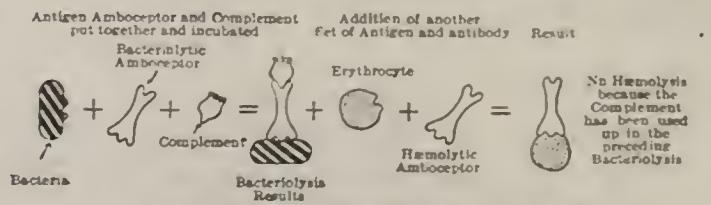


ILLUSTRATION OF PHENOMENA OF BORDET AND GENGOU

A SUMMARY

Natural blood and tissue immunity depends on bactericidal and antitoxic powers of serum and plasma.

Destructive cells as phagocytes on the bacteria and toxin.

Natural blood and tissue immunity depends on bactericidal and antitoxic powers of serum and plasma.

Destructive cells or phagocytes on the bacteria and toxin.

Non-existence of suitable receptors—absolute non-susceptibility.

Unequal distribution of receptors.

Age of patient—extremes are susceptible to pneumonia—especially fatal to alcoholics—Typhoid attacks the middle-aged.

Non-susceptibility—not liable to the disease; immuned to some extent; as the dog will not take measles; lower animals immuned to typhoid (This is not acquired immunity). Family susceptibility, or resistance; as the goat is practically immuned to consumption. Cancer and syphilis are found only in the higher animals. Cancer is common to man after 40 years of age, in skin and glands.

Personal equation, individual resistance or susceptibility.

Transmitted immunity as from the mare to the colt in tetanus.

Antibacterial immunity, (non-antitoxic) produced by inoculations of the toxin or the bacteria. Antibacterial serum kills the bacteria. Antitoxin serum neutralizes the toxin.

Resistance, lowered by hunger, malnutrition, exposure, overwork, and excess of all kinds especially alcoholic.

Acquired resistance to drugs, as morphine, arsenic, et al., is not shown by any anti-drug serum—no immunity to drugs.

The disease depends on larger or smaller doses of the bacteria; thus one typhoid organism to the gallon of water may not be dangerous. It also depends on the lowered resistance.

Law of Specificity—Antibodies produced by one specific kind of bacteria, act only on that same kind of bacteria.

Hemolysis—when foreign red blood cells are injected into animals, it gives the serum of that animal the property of destroying the red blood cells of the foreign animal.

Complement, the substance causing the inactivating of the bacteria.

ANTITOXINS

The word means: Anti—against, and toxin—poison. It is the serum obtained from animals immunized to specific diseases.

Antitoxins were first discovered by Roux in 1888, who described the production of soluble diphtheria toxin in cultures of the bacillus in broth; and in 1890 Von Behring found that antitoxins were formed in the blood of persons suffering from diphtheria. In 1891 Behring and Wernicke administered the first dose of anti-diphtheria serum to a sick child in Berlin.—Previous to this first administration, the serum had been tried on rabbits. And the antitetanic serum had been tried on horses.

Antitoxins are made by immunizing horses to the specific bacteria causing the disease.

Diphtheria antitoxin is made by injecting into a horse (which has been kept for some time, at least 6 months, and frequently examined for disease—the animal must be absolutely healthy) about 100 units of antidiphtheric serum, subcutaneously. This injection is then followed by an injection of a relatively small dose of the toxin (0.002 mils, which is the M. L. D. for the guinea pig). This toxin has been obtained by growing the diphtheria bacillus on blood agar, washing off the growth with sterile salt solution and planting the bacillus in large flasks filled ¾ full of blood veal broth. These flasks are incubated and after the bacillus has grown, the culture is filtered through unglazed porcelain filters, to remove all the bacilli, so that the filtrate contains only the toxins or waste products from the bacilli. This toxin is then standardized by testing on guinea pigs of 250 grams weight. One unit of the toxin is that amount which when mixed with one government standard unit of antitoxin will cause the death of the guinea pig in 4 days, this is the toxin unit or the lethal or L. plus dose.

In a short time, usually several days after the first injection into the horse, after all reactions and the fever have abated, a still larger dose of the toxin is injected, and this is repeated for some time, one to two months, until the horse shows a sufficient number of antitoxin units (300 or more to the mil)—when the horse is said to be immune. The horse is bled by tapping the jugular vein and the blood is drawn into a sterile bottle or very large test tube, containing a small amount of sodium citrate to prevent coagulation. It is then cooled for 24 hours, in the ice box, when the plasma

will have separated from the corpuscles. Seven to 8 liters may be drawn from a horse at one time without injury. This may be repeated several times at sufficient intervals for the horse to recover from the blood drawing. The serum contains the antibodies and is filtered through a Berkefeld filter to remove all bacteria and other cells. It is standardized by mixing various amounts of this serum with 100 times the minimum fatal dose (M. F. D.) of the toxin and the smallest amount of antitoxin which will protect the 250 gram guinea pig against 100 times the M. F. D., or L. dose is called a unit. The antitoxin is tested for sterility, placed in syringes and labeled as to the kind, the number of units, the Government license number under which it was manufactured and the expiration date.

Antitetanic serum is made in practically the same way as the antidiphtheria serum.

Globulins are the active antibodies, and have many advantages. They will keep indefinitely and by mixing with sterile water are ready for use. They cause less anaphylaxis than the serum.

Globulins were discovered by Gibson in 1904. The following is the process: Heating the antitoxin to 50 degrees C. for 15 hours; then diluting with 1 to 3 volumes of water and precipitating the antitoxin molecules or globulins with ammonium sulphate; filtering through paper to separate the albumen and other soluble constituents. And the globulins are again brought into solution by means of sodium chloride —the globulins are then precipitated by dilute acetic acid and separated by filtering and dried by pressing between filter papers. All salts are removed by dialysis and the globulins are brought to the consistency of the blood with normal salt solution, placed on ice for 14 days, filtered through clay filters and dried. Other methods have been discovered, such as the antitetanic globulins, when 17% ammonium sulphate is added to the antitoxin, then centrifuging, and adding 1 to 3% more ammonium sulphate, the precipitate is dried in vacuum.

The LETHAL (L) dose of a toxin is the smallest amount necessary to kill, and it will exactly neutralize the standard unit.

The amount of toxin which when mixed with one unit of standard antitoxin is just sufficient to kill the guinea pig in 4 days is the L. plus.

The smallest amount of antitoxin which when mixed with

the lethal dose will prevent the death of a guinea pig in 4 days, is a unit.

The antidiphtheric unit is that amount of antitoxin which will protect a 250 gram guinea pig against 100 times the L. plus dose.

The M .F. D. is the smallest amount of toxin required to kill the guinea pig.

The antitetanic unit is that amount of antitoxin which will protect a 350 gram guinea pig against 1,000 times the L. plus dose.

VACCINATION AGAINST SMALL POX

The word vaccination comes from vacca, meaning cow. Vaccination against small pox was known to the ancients and probably practiced before the birth of Christ. Vaccination against small pox was known to the ancients and probably practiced before the birth of Christ, by the Chinese, followed by Turkey, India and Mexico, and secretly practiced in Italy for many years. It was brought to Europe in 1718 by Lady Montague; but was not the vaccination used today, but consisted in the inoculation of the virulent virus from the pustules of the small pox patient or from sores of the arm of a vaccinated person—the arm to arm method, or by the scab method, where the scab was carried in the physician's pocket; and when vaccination was required a portion of the scab was rubbed into the scarified arm. By these methods a mild type of the disease, or "variola inoculata" resulted.

In 1789 Jenner noticed that the milk maids of Holland did not contract small pox and investigating found that they had "cow pox," a disease which produced sore udders on the cow and sores on the milkers' hands. Jenner vaccinated with virus obtained from these sores on the udders and prevented in the vaccinated small pox which at that time was the world's plague. For at least 50 years after Jenner's discovery this method of vaccination held sway, that is vaccinating from patient to patient; diseases such as syphilis and others, were communicated in this way. From the time of Warlemont in 1868 this cow pox vaccine which is the attenuated living organisms, has been the universal vaccine.

The vaccine is made by using calves or heifers of from 6 to 12 months of age—washed with soap and water—the abdomen shaved and the virus from another calf is rubbed into scarifications on the shaved abdomen; in about 5 to 7 days, vesicles appear which are the typical umbilicated cratered small pox pustules. The virus taken from one calf is sufficient for 2,000 to 15,000 vaccinations in man. This virus is scraped from the pustules and is ground with 50 to 60% of glycerin, which is a preservative and increases the bulk. This ground virus is placed in cold storage for 6 to 8 weeks and then filtered, and is ready for use. It was formerly marketed on ivory points, but on account of the great liability of contamination this method has been abandoned. The virus dried on these points usually retained it viability for 2 to 4 months, if kept in a cool dark place. Glycerin is supposed

to kill all bacteria except the organisms causing small pox; however certain bacteria thrive and others remain viable in glycerin, for indefinite periods. Pure vaccine is almost impossible to be produced in a commercial way, and the contamination is the cause of the sore arms. These contaminating organisms produce the large sores—a form of blood poison along with rapid destruction of the tissues. Properly prepared vaccine produces only a typical small pox vesicles, and not the large sores usually seen, which come from contaminated vaccine or improper vaccination.

Vaccination usually protects for from 6 to 10 years, when revaccination must be performed. Vaccination will not absolutely protect man from small pox but the disease is always of a light or mild form, and death rarely results, the patient has fewer pock marks, even if the patient has been vaccinated years before. So from this it is seen that at least a small part of the immunity is very lasting.

The only way small pox vaccine is marketed today, is in capillary tubes. The glycerinated vaccine is drawn into these capillary tubes and the ends sealed, thus preventing outside contamination.

On account of the immunity conferred upon the calf, the calf can be used only once for the production of small pox vaccine.

Many authorities claim that revaccination must take place every 7 years. As soon as possible after the babe is born it should be vaccinated.

Small pox vaccine and the antitoxins and bacterial vaccines must be kept in the dark, and in a cool place, such as the ice box. Small pox vaccine will remain viable for a long time if kept at 0 degrees C. It is rapidly affected by heat, and is killed or rendered inert at 60 degrees C. It rapidly deteriorates at room temperatures.

Small pox vaccine should be introduced just beneath the epidermis so as to be absorbed by the lymphatics and capillaries. In scarification, care must be used in not washing the arm with antiseptics and also not to scarify so that bleeding occurs. If the vaccine is viable and applied in the proper manner, there will be no failures but all will be "takes." When vaccination fails it may be the fault of the unskilled operator, or insufficient rubbing of the vaccine, washing out or away of the vaccine, by bleeding, or a dead vaccine.

The word quarantine comes from the Italian—quaranta and means forty.

In general, any infectious disease characterized by skin lesions, may be regarded as infectious or contagious, at least until all scabs or scales have disappeared—such as scarlatina, small pox, etc.

Any disease whose lesions are practically confined to the mouth, respiratory or digestive tracts, would be most liable to conveyance by sputum, eating utensils, vomit, dejecta from bladder and bowels—consumption, syphilis, cholera and typhoid fever.

The Boards of Health of each state have rulings in regard to quarantine and disinfection.

In addition to the Pennsylvania rulings add to section 19, on April 6, 1919, the following:

BE IT RESOLVED THAT all services held in connection with the funeral of the body of any person who has died of measles, mumps, German measles, and whooping cough, shall be private and the attendance thereat shall include only the immediate adult relatives of the deceased, who may at the time not be under absolute quarantine restrictions, and the necessary number of adult pall-bearers; and any advertisement of such funeral shall state the cause of death.

BE IT FURTHER RESOLVED THAT the body of a person who has died of any such disease shall not be taken in any church, chapel, public hall or public building for the purpose of holding funeral services.

Resolutions authorizing the placarding and quarantining of contacts of certain communicable diseases.

BE IT RESOLVED THAT where persons are known to have been exposed to diphtheria, scarlet fever, or small pox, health authorities may, when in their opinion it is necessary, placard and quarantine the premises, using the following form for the placard:

WARNING—SCARLET FEVER:

DIPHTHERIA, SMALL POX

An inmate of this house is known to have been exposed to scarlet fever (diphtheria, small pox) and is required to remain on the premises until released by the health authorities.

Also at the termination of the quarantine period or upon

death or removal of a case of anterior poliomyelitis, German measles, glanders (farcy), measles, mumps, typhoid fever, paratyphoid fever and whooping cough disinfection shall be performed as follows:

The room or rooms occupied by the patient shall be subjected first to a chemical cleansing followed by application of a solution of one to one thousand bichloride of mercury (corrosive sublimate) or a solution of two teaspoonfuls of creolin to a gallon of water.

When the health officer establishes quarantine on a premises for any of the above mentioned diseases, he shall fully instruct the householder regarding the requirements to be observed by all persons under quarantine, and shall advise him of the date upon which quarantine may be raised if no further cases develop. He shall direct that when the quarantine period has expired the householder shall proceed to cleanse and disinfect the room or rooms occupied by the patient, according to the circular on sanitary cleaning which the health officer shall furnish to the householder.

At the termination of the legal quarantine period or upon death or removal of the patient the health officer shall visit the premises and if he finds that the sanitary cleansing has been accomplished as required he shall remove the placard and terminate the quarantine.

In Pittsburgh the diseases to be reported to the Department of Health are: Actinomycosis, Anthrax, Acute Anterior Poliomyelitis, Burbonic Plague, Cerebro-spinal meningitis, Chickenpox, Cholera, Diphtheria (Membranous Croup), Epidemic Dysentery, Erysipelas, German Measles, Glanders, Impetigo, Leprosy, Malarial Fever, Measles, Mumps, Opthalmia Neonatorum, Para-typhoid Fever, Pellagra, Pneumonia (true), Puerperal Fever, Rabies, Relapsing Fever, Scabies, Scarlet Fever, Smallpox, Tetanus, Trachoma, Trichiniasis, Tuberculosis (specify form), Typhoid Fever, Typhus Fever, Uncinariasis, Whooping Cough, Yellow Fever.

The diseases to be quarantined are: Anthrax, Bubonic Plague, Cerebro-spinal Meningitis (epidemic), (Cerebro-spinal Fever, Spotted Fever), Chickenpox, Asiatic Cholera, Diphtheria (Diphtheritic Croup, Membranous Croup, Putrid Sore Throat), German Measles, Glanders (Farcy), Leprosy, Malarial Fever, Measles, Relapsing Fever, Scarlet Fever (Scarlatina, Scarlet Rash), Smallpox (Variola or Varioloid), Typhoid Fever, Typhus Fever, Whooping Cough, or Yellow Fever.

Diphtheria quarantine is released after two weeks, in case antitoxin has been used and two successive nose and throat cultures taken 48 hours apart are negative.

Diphtheria Carrier released after one negative culture.

Scarlet Fever. Four weeks, in case desquamation has caused no ear or nose discharge.

Measles and German Measles. Sixteen days, in case of complete recovery.

Whooping Cough. Five weeks, in case paroxysmal stage has terminated.

Chickenpox. Sixteen days, in case all swelling has disappeared.

Smallpox. Until recovery and until lesions have healed.

Typhoid Fever. Until recovery and until urine and feces are free from bacilli.

Influenza and Pneumonia. After recovery.

The American Public Health Association Rules on Communicable Diseases

1. Identification of infective agent.
2. Source of infection.
3. Mode of transmission.
4. Incubative period.
5. Period of communicability.
6. Method of control.
   a. Infected individual and his environment.
      1. Recognition of disease.
      2. Isolation.
      3. Immunization.
      4. Quarantine.
      5. Concurrent infection.
      6. Terminal disinfection.
   b. General measures.
   c. Epidemic measures.

Rabies (see page on), Tetanus and general diseases are usually contracted by inoculation or close contact.

Mild forms of diseases are just as contagious to others as the most virulent forms; scarlet rash and scarlet fever are the same; likewise, varioloid, however mild, is small pox.

Membranous croup and diphtheria are best regarded as the same.

As soon as suspicion is aroused, the patient should be removed to a sunny, well ventilated room, devoid of curtains,

portieres, carpets, pictures, furniture, clothing, books, toys and everything not absolutely necessary.

The floors should be perfectly bare or covered with bed sheets, plain wooden chairs, but only as many as necessary; a cot for the nurse; no cooking utensils to be kept in the room. Temperature of the room should be about 21 degrees C. For the attendants gowns and caps should always be worn in the sick room.

After quarantine, all things used in the sick room should be burned; if this is not possible, disinfection should take place. (See pages on disinfection). No more articles than absolutely necessary should be in the rooms.

Antiseptic gargles, nasal douches and antiseptic solutions for hand and face as 1/4% phenol in alcohol should be placed near the door; urine and feces disinfected by 1 part of fresh (not slacked) lime to 2 parts of the dejecta. Or equal parts of phenol may be added to the dejecta. These must be in contact for 1 hour before being thoroughly disinfected.

Wood work, furniture, et al., washed with 1 in 1,000 bichloride solution, followed by a washing with soap and water.

The floors should be mopped with cloth moistened with disinfectants, such as bichloride (1 gram to 1 liter of water); or kerosene, which is very good. The bed linen and personal linen with the least agitation should be placed in pillow slips and boiled thoroughly before removing contents.

Cooking utensils should be well sterilized—food remnants and all discharges from the patient should be burned, the discharges being especially dangerous in certain diseases. Linen should be immersed in 5% phenol or other disinfectants for 1 hour, boiled and hung in the sun to dry.

Vaccination, prophylactic injections, bacterial vaccines should be used for their respective diseases.

Spittoons should contain 1 in 1,000 bichloride solution, or other disinfectant solutions for 5 hours contact to kill the bacteria.

Closets must be daily flushed and washed with disinfectants.

After quarantine the patient should be given an antiseptic bath with such as 0.5 gram of bi-chloride to 1 liter of water, washed well with soap and water and a sterilized change of clothing. Nose and mouth washes are of great value. Burn all papers and books which have been in the room, also all toys and unnecessary articles.

Disinfect the room with 1½ lbs. sulphur for each 1,000

cubic feet—remember the sulphur must be burned over water vapors, or 90 to 360 grams of formaldehyde may be used. (See pages on disinfectants).

The milk man should pour into vessels standing outside the house, without touching the vessel; use no return bottles, as milk is an excellent food for bacterial growth.

Cats, dogs, birds and rats, et al., carry diseases as pneumonia, consumption, diphtheria, etc.

No letters from diseased persons or quarantined houses should be mailed; scarlet fever has been carried in this way.

Persons dying of contagious diseases should be wrapped and placed in sealed air tight lead or iron caskets. Masks should be worn by the nurse; these masks can be made as follows:

The gauze (44 by 40 mesh) is cut 8 inches wide and 23 inches long. The sides and one end are turned down one-quarter inch. It is folded twice, the unturned end first, making a 7½ inch square. The opposite diagonal corners are cut off 1 inch and the raw edge is turned in one-half inch. It is stiched firmly all around. A 1-inch dart 1½ inches long is taken up at the middle of each side of the mask. A 14-inch tape is sewed on the opposite uncut corners. This mask has the advantage of covering the nose and mouth and in making the traction on the chin and not drawing on the nose and lips.

During ordinary or loud talking infected material from the mouth may be projected to and through the danger zone of 10 feet from the patient.

During coughing infected material is easily projected 10 feet from the patient.

Masks of coarse or medium gauze of from 2 to 10 layers do not prevent the projection of infected material from the mouth during coughing. A 3 layer butter cloth mask is fairly effective, preventing the projection of infective material.

Taliferro claims that 386,030 deaths occur annually from spray borne diseases.

Cholera, typhoid and diphtheria and similar diseases (see pages on) may be carried by healthy persons indefinitely and may infect other persons—although the carriers are seemingly well, they are dangerous.

For external use, the iodine solution of formula previously given, or iodine 1 gram and potassium iodide 2 grams and glycerine 60 mils is efficient.

Phenol is dangerous, often causing gangrene.

Savage's classification:

A. Gaertner group—a sub-group of coli-typhoid group, between the chemically active typhoid and the inert coli group.
   1. Bacillus enteritidis—any of the Gaertner group, strains of the B. typhus murium.
   2. Bacillus suipestifer—associated with B. typhi murium groups, probably B. psittacosis.
   3. Para typhosus groups.

B. Non-Gaertner group, aerobic groups—such as B. proteus, B. coli.

C. B. botulinus—sausage, ham, etc.—Caused by improper curing. They are destroyed by heating and cooking. This group will not grow in media containing more than 6% of sodium chloride; hence if sufficient (15%) salt is used in curing the meat, no growth will occur.

Vaughn and Novey's Classification:

1. Food poisoning—Bromatoxismus.
2. Milk poisoning—Galactoxismus.
3. Cheese poisoning—Tyrotoxismus.
4. Meat poisoning—Kreatoxismus.
5. Fish poisoning—Ichthyotoxismus.
6. Mussel poisoning—Myhlotoxismus.
7. Cereal poisoning—Silotoxismus.

Donk isolated a thermophilic organism from cans of "flat sour" corn. Pure cultures introduced into sterile cans of a variety of canned foods (corn, peas, string beans, pumpkin and tomatoes) produced the same characteristic "flat sour." The following organisms were identified: M. acidi lactici in cheese; M. candicans in roast beef, sardines, and bulk granulated sugar; M. candidus in baked beans; M. cereus in baked beans; M. lactis in cheese; M. luteus in corn; M. pyogenes in two samples of Maine style corn and one sample of canned corn on the cob; M. stellatus in canned roast beef; B. cloacae in canned roast beef; B. detrudens in cheese; B. licheniformis in string beans; B. megatherium in sauer-kraut brine (not canned) and cheese; B. mesentericus in cheese; B. pammellii in cheese; B. subtilis in corn; B. tenuis in cheese; B. viscosus in cheese; B. vitalis in spinach and in bulk granulated sugar; B. vulgatus in two samples of corn; Bact. welchii in corn.

KOCH'S LAWS

In order that a disease may be traced and proven to have been caused by a specific bacterium, Koch's laws must be observed, which are:

1. The organism must be constantly present in the proper tissues of animals suffering, or dead from the disease.
2. The organisms must be cultivated artificially in pure cultures.
3. The disease must be reproduced in suitable animals from these pure cultures.
4. Organisms must be again cultivated in pure cultures from the tissues of the experimental animal—clinical symptoms, et al., confirming.

OBSTACLES TO THE DISCOVERY OF MICRO-ORGANISMS

1. Organisms too small to be seen under the microscope (Microscopes not perfect or powerful enough to distinguish causing organisms).
2. Inability to stain the organisms.
3. Cannot cultivate artificially.
4. Will not cause the disease in animals—cannot produce the same disease in animals—as man has typhoid fever and animals will not take typhoid fever.

Gruber-Widal reaction, gives valuable evidences of reactions of disease as typhoid fever, epidemic dysentery, shingles, et al. The complement fixation test, and Abderhalden's reaction are also of great importance.

It is not necessary to fulfill all of Koch's laws; in some diseases as in typhoid fever, or malaria, where the Anapheles, the mosquito, carries the infection, and the patient is the host. Malaria epidemics have been killed by frost.

Mosquito control.—For one acre of mosquito breeding ground and water, use 26 bushels of saw dust (1 bushel to gas oil 3 gallons), thoroughly mixed and scattered broadcast.

Specific infections are diseases caused by one certain kind of bacterium, which always produces characteristic clinical symptoms and anatomical (Autopsy) lesions.

Many diseases of unknown etiology, as Colds, Scarlatina (same as scarlet fever but a mild form); it is supposed

that scarlet fever is caused by a streptococcus, and that Typhus is caused by a protozoon.

Rubella, or Rubeola—German measles, a form of which is Morbelli, which may be fatal, and if not fatal, leaves the patient weakened, as very susceptible to pneumonia, etc., while Rubella is never fatal and has few complications.

Many diseases are caused by a filterable virus. 38 or more known diseases are attributed to this cause, as Foot and mouth disease, Vaccinia, Variola, Measles, Yellow fever, Scarlet fever, Molloscum contagiosum, Denuge-demige fever, Sand fly or 3 day fever, Polymyelitis, Typhus and others.

Filterable viri are porcelain filtrates of the exudates from certain diseases. They are strongly toxic substances, or living ultra-microscopic filterable organisms, unculturable and having great infectiousness, producing active immunity. They are of a wide geographic distribution, being disseminated with ease and rapidity and have a high pathogenic power in high dilutions. They are all invisible and cannot be artificially cultured. They resist ordinary methods of disinfection to a great extent, surviving drying for some time and are transmitted by air, water, vermin, etc.

Symbolic infection exists when the patient has two distinct diseases at the same time, as measles and scarlatina.

Non-specific diseases are those which may be caused by various bacteria, as boils, carbuncles, septicemias—blood poison, membranous croup, bronchitis, throat, lungs and joint infections arthritis, which may be local and general, endocarditis, inflammation of the lining of the heart, etc.

Mixed infections occur when two or more bacteria are present, or when the patient may have for example measles and diphtheria with scarlatina, chanchers hard and soft with gonorrhea, diphtheria and a streptococcus infected throat, Tetanus bacillus and acrobes, diphtheria and infantile paralysis.

DEATHS FROM INFECTIOUS DISEASES

The figures in the following tabulation are from mortality statistics published by the Bureau of the Census of the U. S. Department of Commerce:

Deaths in Registration Area of United States, 1911-1916*

	1916	1915	1914	1913	1912
Tuberculosis:					
All forms	101,396	98,194	96,903	93,421	90,360
	(141.6)	(145.8)	(146.8)	(147.6)	(149.5)
Lungs	88,666	85,993	84,366	80,812	78,465
	(123.8)	(127.7)	(128.8)	(127.7)	(129.9)
Meningitis	5,706	5,445	5,410	5,467	5,098
	(8.0)	(8.1)	(8.2)	(8.6)	(8.4)
Other forms	7,024	6,756	7,127	7,142	6,797
	(9.8)	(10.0)	(10.8)	(11.3)	(11.2)
Pneumonia:					
All forms	98,334	89,326	83,804	83,778	79,917
	(137.3)	(132.7)	(127.0)	(132.4)	(132.3)
Bronchopneumonia	35,105	33,501	32,153	31,094	28,422
Lobar	54,699	44,153	33,911	32,615	30,003
"Pneumonia"	8,330	11,672	14,740	20,069	21,492
Scarlet fever	2,335	2,419	4,340	5,498	4,038
	(3.3)	(3.6)	(6.6)	(8.7)	(6.7)
Measles	7,947	3,649	4,461	8,108	4,240
	(11.1)	(5.4)	(6.8)	(12.8)	(7.0)
Whooping cough	7,284	5,421	6,816	6,332	5,619
	(10.2)	(8.1)	(10.3)	(10.0)	(9.3)
Diphtheria and croup	10,367	10,544	11,786	11,920	11,013
	(14.5)	(15.7)	(17.9)	(18.8)	(18.2)
All causes	1,001,921	909,155	898,059	890,848	838,251
	(1,398.9)	(1,350.2)	(1,360.9)	(1,407.4)	(1,387.2)

*Figures in parentheses are rate per thousand population (from "Mortality Statistics," Bureau of the Census, Department of Commerce, Washington, D. C.).

THE GROWING AND ISOLATION OF MICRO-ORGANISMS

A culture is a growth of planted micro-organisms.

A pure culture is a growth of only one kind of organism.

The artificial food for growing micro-organisms must contain water, food in an easily digested form, neutral or feebly alkaline (except for molds, which must be feebly acid). They must be sterile and represent as nearly as possible the natural food of the micro-organisms. The artificial

food may be liquid, solid or semi-solid.

The base of all foods is the nutrient bouillon or broth, which is composed of

Meat extract	5 grams
Pepton	10 grams
NaCl	5 grams
Water qs	1 liter

These substances are dissolved in a vessel containing 1 liter of tap water, the vessel is weighed, then heated until all the substances are dissolved, again weighed, and by adding water, the weight is made up to replace that lost by heating. The medium is then titrated and made neutral by adding normal sodium hydroxide or in some cases sodium phosphate. Heat for 10 minutes. The medium is now filtered through paper and placed in sterile flasks or test tubes. These flasks and test tubes should be plugged with cotton; the cotton plug should be wrapped in gauze to prevent sticking to the sides of the container. The cotton permits free access of air but filters out all the organism in the air.. If the medium cannot be cleared by filtering, it may be cleared by adding the white of two eggs to the L. of the medium. Stir the whites of the eggs in water, then add this mixture to the medium; constantly stirring, heat for 5 minutes and filter. Sterilize. If meat infusion broth is desired, use 500 grams of lean meat, in place of the meat extract. Cut the meat in fine particles, or grind, add 1 liter of water, and place in the ice box for 12 hours, strain through cheese cloth, add water to make up 1 liter, then add the salt and peptone and proceed as above directed.

Gelatin medium. Add to 1 liter of broth medium, 120 grams special bacteriological gelatin, warm to dissolve, titrate and adjust; add the whites of two eggs as directed under broth media; warm on the water bath for 30 minutes; stir thoroughly and again heat for 15 minutes; adjust the weight by adding water; filter through cotton and sterilize by fractional sterilization, never in the autoclave; for prolonged or tco high heating will spoil the solidifying properties of gelatin.

Agar medium. Add to 1 liter of broth 8 grams of agar, dissolve over heat, make up the water lost by evaporation, titrate and adjust, to 0.5 acidity, clear with the whites of eggs and again heat; if in the autoclave for 15 minutes at 15 lbs. pressure, or in the sterilizer as the Arnold for 30 minutes. Again titrate and adjust. Heat and filter through cotton and tube and sterilize.

Litmus-Lactose Agar is made by adding to the nutrient agar medium 1% lactose and sufficient litmus to give the medium a purple color when cold.

Dorset egg medium, used especially for the cultivation of the tubercle bacillus, is made by mixing the whites and yolks of 4 eggs, adding 25 mils of water and mixing, and filtering through sterile cloth. Ten mils are placed in each sterile test tube and the tubes placed in a water bath at 45 degrees C. for 10 minutes to expel the air from the egg mixture; the tubes are plugged with cotton and sterilized by heating the slanted tubes for 5 hours a day for 2 days at 70 degrees C. The third day the sterilization is finished in the Arnold. Before inoculating these tubes, 3 drops of sterile bouillon should be added to each tube; and 5% glycerine is also added for the cultivation of the human type bacilli.

Glycerin agar is made by adding 5% glycerin to the nutrient agar medium.

Ascites agar is made by adding sterile ascites fluid to nutrient agar. This fluid is taken from a patient suffering from dropsy and then sterilized.

Nutrient sugar broth is made by adding 1% of the required kind of sugar to the nutrient broth.

Sugar free broth is made by inoculating 1 liter of nutrient broth with Bacillus coli communis and incubating for 2 days. The sugars are fermented out by the coli bacillus. They are then killed by sterilization, and filtered out. Titrate and adjust to 0.2 acidity. Heat throughly and filter through paper or the egg whites may be used. It is now divided into 250 mil portions and the desired sugar may be added, usually about 1%, and sterilized, not over 20 minutes at a time.

Starch agar, is made in the usual way for nutrient agar, except salt and peptons are omitted—it is adjusted to 0.5 acidity and 10 grams of corn starch to the liter is added—Cook in the autoclave for 30 minutes at 10 lbs. pressure, tube and sterilize.

Loeffler's Medium. Blood serum obtained by collecting beef blood in tall cylinders and under sterile conditions, cooling in the ice box for 24 hours and separating the serum from the clot, is added to veal or beef bouillon in the proportions of 1 part to 3 of the serum.—Tubed and warmed as in the egg media to drive out the air, and sterilized—Or the serum may be added to nutrient agar.

Serum water for fermentation tubes. Hiss's medium. This is used for determining the sugar-splitting powers and acid formation of various bacteria. Beef or sheep serum is

diluted with three times its volume of distilled water, heated in the Arnold for 15 minutes, and 1% of the various sugars which it is desired to use, added and sufficient litmus to give it a deep purple color. Sterilize by the Arnold. When this medium is used for pneumococcus and streptococcus differentiation, inulin dissolved in water is added, and then sterilized in the autoclave. The serum is then diluted with this sterile inulin solution and sterilized as usual.

Sodium glycocholate agar—or MacConkey's medium, consists of 2% nutrient agar to which 1% lactose and 0.5% sodium glycocholate are added.

Anaerobic Methods

Hydrogen Displacement Method.—Consists of generating hydrogen in a Kipp apparatus passing it through a series of Woulfe bottles containing lead acetate and pyrogallic acid respectively—and also one with silver nitrate to take out any hydrogen arsenide which may have come from impure zinc. If a Novy is not obtainable, a wide mouth bottle may be used.

Nitrogen gas may be used instead of hydrogen, being obtained from the cylinders of compressed nitrogen.

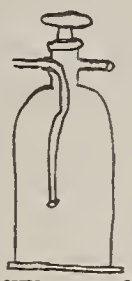
Pyrogallol Method. Large test tubes about 2.5 by 26 cm. are used. In the bottom is placed about ½ teaspoonful of pyrogallol; this is packed down with a small amount of cotton.—Gently flow over the cotton about 15 mils of a 20% potassium or sodium hydroxide solution and immediately slip the inoculated culture tube into the larger tube, so that it rests on the cotton in the bottom, and stopper the large tube tightly with a rubber stopper.

1. INNER TUBE IN POSITION INVERTED OVER WOODEN ROD.
2. AFTER BEING HEATED IN THE AUTOCLAVE, THE INVERTED TUBE BECOMES FILLED WITH THE MEDIUM.

1. 2.

GRAVE'S MODIFICATION OF THE DURHAM FERMENTATION TUBE.

Another method is to place the inoculated culture tubes n à dessicator, the lid of which is supplied with a stop cock. Place a piece of alcohol saturated filter paper into the dessicator and light it. Quickly replace the well vaselined lid, close the stop cock and a vacuum will be formed. It will be necessary to open the stop cock before the cover can be removed.



NOVY JAR FOR
GROWING ANAEROBES.

OBTAINING A PURE CULTURE OF BACTERIA

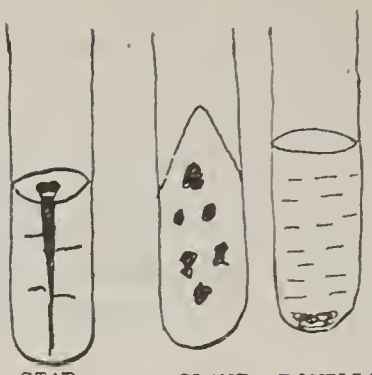
When broth cultures are made from substances, as sputum, urine, feces, soil, water and milk, many kinds of organisms develop simultaneously side by side and a heterogenous mixture of bacteria results.

To obtain one kind of bacterium only, test tubes with gelatin or agar medium are placed in a water bath and melted, at a temperature just above the melting point. One tube is inoculated with a loop full of the broth culture or they may be inoculated from fresh material such as pus, or if the pus or other material is too heavy or thick, it may be diluted by mixing in a test tube with sterile salt solution and inoculated. The inoculation is made by removing the cotton plug with the third and fourth finger of the right hand, and holding the plug between these fingers; do not lay it down; then heating the platinum needle red hot in the flame, cooling, and placing one loopful of the material in the tube of melted medium. The needle is immediately flamed, then the plug flamed and placed in the tube. The contents of the tube are carefully mixed by tilting back and forth and also rotating the tube. When the contents have been thoroughly mixed, inoculate another tube with two loop fulls of the mixture of bacteria and medium. Carry out the same process in regard to the inoculation, flaming of the needle and handling of the plugs. Mix thoroughly, and inoculate a thrid tube with two loop fulls from the second tube; mix thoroughly. The tubes should be placed in the water bath after each inoculation, to prevent the medium from hardening. The tubes having been inoculated, three dilutions are formed. Petri dishes are inoculated from these tubes, by removing the plug from the tube and holding in the hand; flame the lip of the tube, and raise one edge of the lid of the sterile Petri dish just high enough to permit the open end of the test tube to be inserted, pour the medium and bacteria into the Petri dish, replacing the lid and tipping the Petri dish so that the medium is evenly distributed before it solidifies. This process is called seeding or plating out. Incubate when cold.

If there are a great number of bacteria in the original material, plate No. 3 will contain the organisms in sufficient numbers to develop typical colonies; but if few bacteria are present then plate No. 1 will contain the most typical colonies. In giving a report, as in water or milk analysis, each colony contains an innumerable number of bacteria; so the report should be so many colonies or points of growth to the mil, and not so many bacteria to the mil.

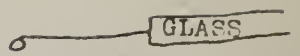
Petri dishes should be turned upside down, when placed in the incubator. This is to avoid the water of condensation from dripping down onto the medium, which may cause spreading of the colonies.

Placing the bacteria in a solid medium prevents them from moving about as in the liquid medium; hence they are fixed at one point and pure cultures result. These plates must be examined at least every 8 to 12 hours, and when the colonies have sufficiently grown, they are touched with the end of a platinum needle; this is then inoculated into other culture media, and pure culture is obtained. This process is called fishing.

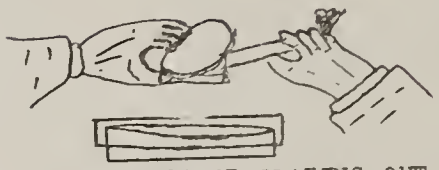


STAB SLANT BOUILLON

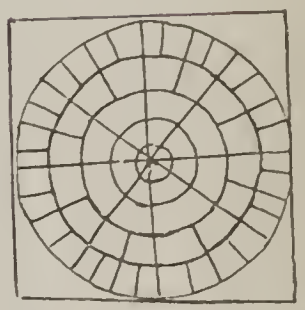
METHODS OF CULTURING.



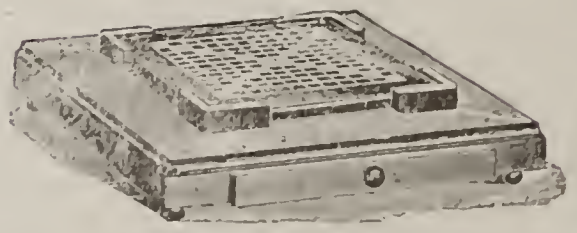
PLATINUM NEEDLE. THE LOOP IS MADE AROUND THE LEAD IN A LEAD PENCIL



'SEEDING' OR PLATING OUT IN PETRE DISHES.



RULED GLASS PLATE FOR COUNTING THE NUMBER OF COLONIES OR POINTS OF GROWTH IN PETRE DISHES.



Board for counting the number of bacterial colonies

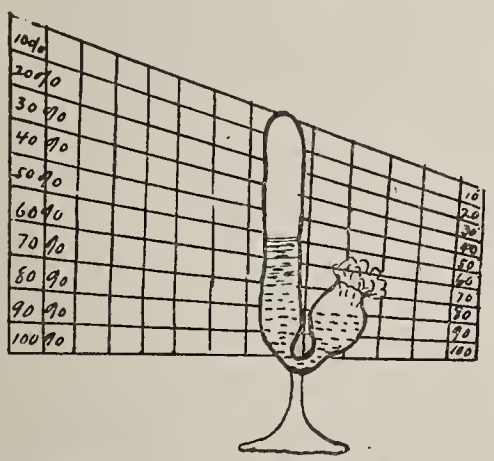
DETERMINING THE PRODUCTION OF INDOL

The organisms are inoculated into tubes containing Dunham's solution, which consists of 1% peptone, 0.5% sodium chloride in water. After incubating at 37 degrees C. for 4 days, two drops of concentrated sulphuric acid and 1 mil of 0.01% solution of sodium nitrate are added; and after 30 minutes, the tube is slightly warmed if no color has developed in this time. A pink color indicates indol.

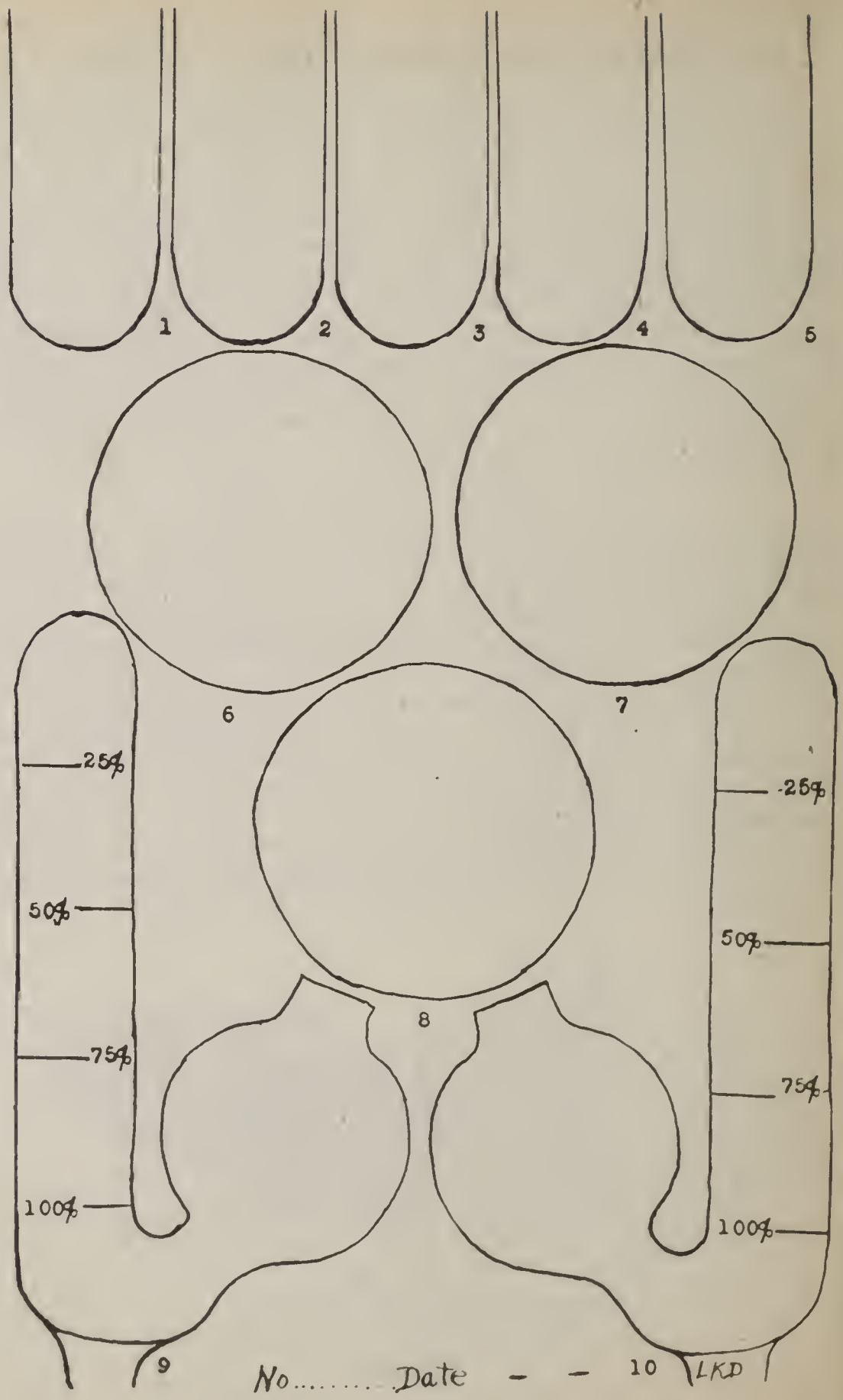
Or to 10 mils of the suspected culture add 5 mils of: paradimethylamidobenzaldhyde 4 parts, absolute alcohol 380 parts and concentrated hydrochloric acid 80 parts; shake thoroughly, then add 5 mils of saturated aqueous solution of potassium sulphate; a red color, becoming darker on standing indicates the presence of indol.

THE FERMENTATION TEST

Some bacteria have the property of fermenting carbohydrates. Special tubes are filled with broth from which the muscle sugar has been removed and 2% glucose or lactose has been added. The medium in the tube is sterilized and inoculated with the suspected material—incubated for 48 hours at 37 degrees C. and examined for gas. The gas has collected in the arm of the tube and the medium is forced into the bulb. The gas may be analyzed by adding a 2% sodium hydroxide solution to fill the bulb. The mouth of the bulb is closed by the thumb and the tube shaken several times, and then the gas is permitted to collect in the arm. As soon as the thumb is released the fluid rises in the arm, on account of the sodium hydroxide having absorbed the carbon dioxide; the residual gas which is hydrogen can be estimated.



CARD BOARD SCALE FOR ESTIMATING GAS IN FERMENTATION TUBES.


1
2
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25%
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No..... Date - -
LKD

CULTURE MEDIA BEST SUITED FOR THE FOLLOWING ORGANISMS

Simple media for Staphylococcus, Streptococcus, Coli and Anthrax.

Human ascitic serum agar, Gonococcus.

Glucose ascitic agar, Streptococcus, Pneumococcus, Gonococcus.

Loeffler's Medium, Streptococcus, Pneumococcus and Diphtheria bacillus.

Glucose sheep serum agar, Meningococcus.

Whole blood agar plates, Influenza bacillus. (Human or pigeon blood).

Deep anaerobic tubes of glucose agar, for Gas bacilli, and Tetanus.

Glucose or glycerin agar for Glanders.

Dorsett's egg medium for Tubercle bacillus. (Or glycerin bouillon of agar).

TABLE OF DISEASES

Disease	Days of Incubation	Caused by	Infectious (I) Contagious (C)	Carried by
Acute polymyelitis.	2 to 14	(?)	(?)	Nasal excretions.
Anthrax.	2 to 7	B. anthrax.	I. and C.	Cattle, man.
Asiatic cholera.	2 to 4	Vibrio cholerae.	I.	Animals, food.
Boils, carbuncles.	(?)	Various bacteria.	I.	Animals, food.
Bubonic plague.	4	B. pestis.	I.	Animals, food.
Diphtheria.	2 to 5	B. diphtheriae.	I. and C.	Animals, food.
Cancer.	Indefinite	Probably a protozoon.	(?)	(?)
Chicken pox.	16	(?)	C.	Animals, food.
Colds.	Indefinite.	Mixed infection. Usually find Pfeiffer's bacillus, influenza bacillus, pneumococcus, staphylococcus, diphtheria group, ozone bacteria, and many others.	I. and C.	Exposure to disease.
Consumption.	Indefinite	B. tuberculosis & various bacteria.	I.	Exposure.
Dysentery.	8	Entameba Histo-	I.	Food.

		lytica.		
Erysipelas.	4 to 6	Streptococcus pyogenes and others.	Very C.	Animals, etc.
Glanders.	3 to 5	B. Malei.	I. and C.	Horses, etc.
Goiter.	Indefinite	Rod shaped organism (?).	(?)	(?)
Gonorrhea.	3 to 5	Diplococcus gonococcus.	I. only.	Contact.
Grippe.	(?)	Mixed infection.	Very C.	Exposure to disease.
Hook worm.	Indefinite	Uncinaria Americana.	I.	Eggs of worms.
Influenza.	1 to 4	B. influenzae and others.	I. and C.	Exposure to disease.
Leprosy.	Indefinite	B. leprae.	I. C. (?)	Exposure (?).
Malaria.	6 to 10	Plasmodia malariae.	I.	Mosquito.
Malta fever.	6 to 10	Micrococcus melitensis.	I.	Foods, insects.
Measles.	8 to 14	(?)	I. and C.	Exposure to disease.
Meningitis.	Indefinite	Diplococcus (?).	(?)	Carriers.
Mumps.	10 to 21	(?)	I. and C.	Exposure to disease.
Pellagra.	Indefinite	Aspergillus (?).	(?)	Spoiled foods.
Pink eye.	Indefinite	(?)	(?)	(?)
Pneumonia.	1 to 2	B. pneumonia and various bacteria.	I.	Exposure.

Rabies.	20 to 60	Plasmodia Leteve.	I. and C.	Rabid animals.
Relapsing fever.	5 to 6	Spirocheta Obermeieri.	I.	Insect pests.
Rheumatism.	Indefinite	Diplococcus (?).	(?)	(?)
Ring worm.	Indefinite	Fungus.	(?)	(?)
Rubella (German measles.)	1 to 14	(?)	I. and C.	Exposure to disease.
Scabies (Itch)	Indefinite.	(?)	(?)	(?)
Scarlet fever and scarlatina.	1 to 7	Probably streptococcus.	I. and C.	Exposure to disease.
Small pox.	12 to 18	(?)	I., very C.	Exposure to disease.
Syphilis.	14 to 30	Treponema pallida.	Very I.	Contact.
Tetanus.	2 to 5	B. tetani.	I.	Infection.
Tuberculosis.	Indefinite.	B. tuberculosis.	I.	Exposure.
Typhoid.	14 to 21	B. typhosus.	I. and C.	Food.
Typhus fever.	4 to 7	(?)	I.	Insects.
Vaccinia.	3 to 6	(?)	I.	Vaccination.
Varicella.	14 to 15	(?)	C.	Exposure to disease.
Whooping cough.	8 to 14	(?)	I. and C.	Exposure to disease.
Yellow fever.	8	(?)	I.	Mosquito.

EXAMINATION OF BACTERIA

Read and observe the following very carefully:

Go slow.

Use great care.

Understand fully what you are about to do.

Think about each step before taking it.

Wear an apron, or laboratory coat, and avoid carrying infection.

Keep your floor and desk area clean.

Place all discarded cultures and slides in receptacles provided for that purpose.

Label everything and do it legibly.

Clean the oil immersion lens with lens paper as soon as you are through with it.

All slides and cover glasses must be cleaned in the potassium dichromate and sulphuric acid solution (see pages on formulas) and preserved in wide mouthed bottles in alcohol.

If a culture is dropped on the table or floor, breaking the glass or spilling it, do not clean it up until everything has been disinfected for one hour, by covering it with the disinfectant and a towel wet with the disinfectant spread over the entire infected area.

Turn your gas burners out when through using them.

Do not take more stains or reagents than you actually need—there are plenty for all, but none to waste.

Never pour any stain or reagent back into the bottles.

Always use filtered stains.

In taking bacterial specimens from a culture of solid medium where the bacteria are growing on the surface, gently touch the surface of growth with the platinum needle. Do not rub the needle over, or into the growth.

STAINING BACTERIA

Bacteria must be stained in order that the form may be plainly seen, and is accomplished as follows:

A. Placing the bacteria on the slide.

1. Place a SMALL drop of water on the center of the slide, or cover glass, which has previously been cleansed as directed, preserved in alcohol and taken from the alcohol by forceps and passed through the flame, permitting the alcohol to burn.

2. Flame the cotton stopper of the test tube containing the culture, by rapidly passing the cotton stoppered end of the tube through the flame and immediately extinguishing by pinching, not blowing on the stopper.
3. Hold the test tube between the thumb and forefinger of the left hand, the lower end of the tube resting on the back of the hand, the tube being held in an inclined position, never horizontally, as this affords an opportunity for micro-organisms from the air to enter the tube. When the tube is held in this manner, it is held securely and a view of the point to be touched by the needle is obtained.
4. Hold the platinum needle in right hand and sterilize in the flame until red hot—flame kills all life.
5. Remove the cotton plug from the tube with the third and fourth fingers of the right hand, and insert the cooled needle into the tube, care being taken not to touch the sides of the tube with the needle, and transfer a measure, or a loopful of the bacteria to the drop of water on the slide or cover glass.
6. AT ONCE flame or sterilize the needle in the flame, then flame the cotton plug by passing it through the flame; plug the tube, pinching out the flame in the cotton plug; do not blow it out.
7. Flame the needle, permit it to cool, then mix the bacteria and water thoroughly.
8. Dry this mixture in the air, protected from the dust.
9. When dry, FIX the specimen—also killing the bacteria, by passing the slide or cover glass, smeared or filmed side up, three times through the flame, at the rate of a pendulum, holding the slide or cover glass in the hand. Heat coagulates albuminous matter, fixing the bacteria to the slide.

B. Staining.

10. Cover the cooled slide, or cover glass with the stain, and permit the stain to remain the exact required time.
11. Wash off the excess stain with distilled and filtered water.
12. Dry with lintless blotters.
13. Place a small drop of water on the stained portion; cover with a clean cover glass; or if the bacteria are on a cover glass, place the cover glass on a slide with a small drop of water; examine and classify as to the shape.

14. If the specimen is good—uniformly and properly stained and not too heavy, dry thoroughly and mount permanently by placing a drop of chloroform balsam on the center of the cover glass, and then placing the cover glass, balsam side down, on the slide, pressing gently to spread the balsam. Label properly as to stain and organism.

THE GRAM STAIN

The gram stain divides the bacteria into two classes: The Gram positive—those which retain the Gram stain and will be stained purple-black. The Gram negative are those which lose the Gram stain and are colorless until stained with the counter stain, when they will be stained according to the color of the counter stain.

1. Proceed as in Nos. 1 to 9 as above directed.
2. Stain 5 minutes—by the watch—with the aniline-gentian-violet solution.
3. Wash off excess stain with alcohol.
4. Treat with Gram's iodine 1 minute, or UNTIL BLACK.
5. Dry with a lintless blotter, and counter stain with 95% alcohol until no more color comes away.
6. Again counter stain with Loeffler's alkaline methylene blue solution, for 5 minutes; or solutions of Bismark brown, or saffranin may be substituted for the Loeffler's, staining 1½ minutes.
7. Wash in water, and carry out exercises 12 to 14.

CLASSIFICATION OF SOME OF THE MOST IMPORTANT BACTERIA BY THE GRAM STAIN

Gram positive—are stained purple-black.	Gram negative—take counter stain.
Micrococcus pyogenes aureus.	Meningococcus.
Micrococcus pyogenes albus.	Gonococcus.
Streptococcus pyogenes.	Micrococcus catarrhalis.
Micrococcus tetragenus.	Bacillus coli.
Pneumococcus.	Bacillus dysenteria.
Bacillus subtilis.	Bacillus typhosus.
Bacillus anthracis.	Bacillus paratyphosus.
Bacillus diphtheriae.	Bacillus fecalis alkaligenes.

Bacillus tetanus.
Bacillus tuberculosis, and other acid fast bacilli.
Bacillus aerogenes capsulatus.
Bacillus botulinus.
Saccharomycetes.
Molds.
Bacillus enteritidis.
Bacillus proteus.
Bacillus mallei.
Bacillus influenzae.
Bacillus mucosus capsulatus.
Bacillus pestis.
Bacillus maligni edematis.
Spirillum cholerae.
Bacillus Koch-Weeks.
Bacillus Morax-Axenfeld.

It is stated that a 1 in 1,000 gentian violet solution will kill the Gram positive bacteria, but will not kill the Gram negative bacteria.

The bactericidal power of CR (China blue-Rosolic acid) is due to the action of rosolic acid.

Almost all gram-negative bacteria grow readily on a medium containing 25 times the amount of rosolic acid which is inhibitive for gram-positive organisms. This selective action of rosolic acid and its failure to inhibit the growth of B. dysenteriae renders this dye particularly suitable for the preparation of selective mediums to be used for the isolation of intestinal bacteria.

The activity of a dye such as gentian violet in the treatment of infections is described as "bacteriostasis," the dye being called a "bacteriostat" and its property referred to as "bacteriostatic." Gentian violet solutions will cause the death or delay growth of Gram positive bacteria more readily than those which are Gram negative.

EXAMINATION OF LIVING BACTERIA—THE HANGING DROP METHOD

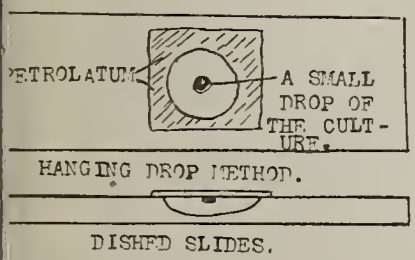
Bacteria must be studied alive in order that the form, motility, and spore formations may be observed. The following precautions must be noted, and the directions followed word for word. And if you are so unfortunate (or careless) as to contaminate the slide or cover glass with bacteria, bring these articles AT ONCE and place them in the receptacle provided for this purpose.

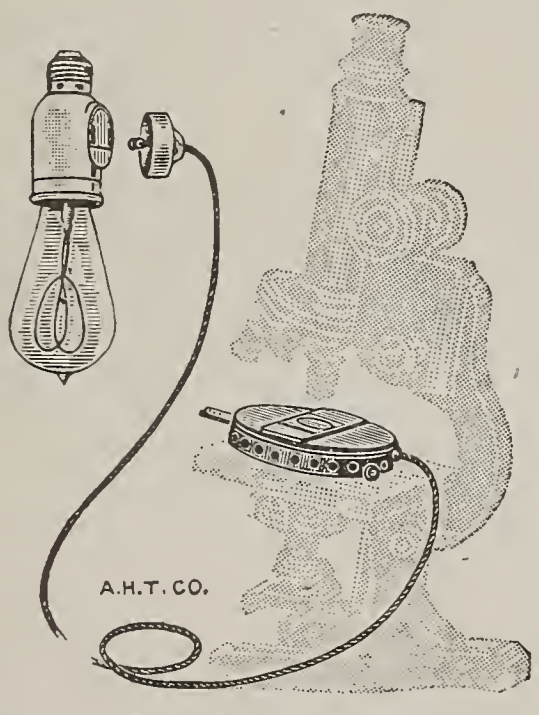
Focus carefully so that the lens will not break the cover ass; if this should happen, call the instructor AT ONCE. se the smallest diaphram opening; then focus the edge of e hanging drop with the low power lens; then exactly cen- r the specimen. Remove the low power and use the high wer lens and focus as you have been instructed, with the e at the level of the stage until the lens ALMOST touches e cover glass, et al.

1. Paint a ring of vaseline or cedar oil around the hol- w, or concave part of the dish slide.

2. Carry out very carefully the exercises 1 to 7 under acing bacteria on the slide. Use a small drop of water; not smear the bacteria all over the cover glass. Place a op of water exactly in the center.

3. Place the cover glass on a smooth part of the table; en invert the vaselined dished slide carefully over the cover





Electric Warm Stage

glass, so that the drop on the cover glass will be in the exa center of the concave part of the slide. The drop shou not come in contact with the slide; if this should happe place slide and cover in the container provided for this pu pose.

4. Gently press on the slide, thus sealing the chamber concave portion, and carefully invert.

5. Examine. Remember the rule in focusing. Do n permit the lens to come in contact with the cover glass.

6. After studying and making your drawings and note remove the slide from the microscope, inverting carefull and insert a teasing needle under one side of the cover gla and remove from slide; if you have carried out this properl the cover glass will be removed with the drop of bacteri emulsion intact and on the upper side of the cover glass.

7. Dry the cover glass in the air and stain as instructe Clean the dish slide and place where it will not be brok for future use.

CHARACTERISTICS OF SOME OF THE BACTERIA

The Staphylococci found commonly in boils, abscesses, arbuncles and similar suppurative processes were first shown y Pasteur in 1880 and by Ogston in 1881. These organisms ccur in aggregates or clusters like a bunch of grapes. They tain with ordinary stains and are Gram positive. Growing t 37 degrees C. and usually are aerobic, and grow upon ordiary media. One of the varieties, staphylococcus aureus, is sually non-pathogenic and may be found on healthy skin. t grows rapidly on blood serum media. Staphylococcus may iquefy gelatin and produce soluble toxins, one of which will estroy leukocytes. It is stated that if the skin is well washd, dried and rubbed to such an extent that irritation results, hat these organisms will be rubed into the skin and produce arbuncles. They have the power of penetrating the skin unided, especially through the weaker portions and may cause oils, etc. Suppurative inflammations in nearly every case ontain staphylococcus. Although a few varieties are nonathogenic, it is best to consider all species as pathogenic. Staphylococcus pyogenes and staphylococcus haemorrhagius re especially pathogenic. Staphylococcus albus is found in eep layers of the skin, which are usually not reached by disnfections and in many operations causes "stitch abscesses." An immunity to a certain extent may be had from the imnunized serums and bacterins.

Streptococci resemble the staphylococci, in many respects, eing discovered by the same men in the same years. With ew exceptions they are pathogenic. They appear as a chain ike formation as seen in Streptococcus erysipelatis and treptococcus pyogenes. And like the staphylococcus they are ot motile. Growing best in dextrose media, they do not ordinarily liquefy gelatin. The varieties are differentiated on lood agar plates and usually curdle milk. Some of the more ommon are S. mucose (which are capsulated), S. viridans, S. conglomeratus, S. brevis, S. longus and S. haemolyticus. The virulence varies with the species and passage through nimals. No specific toxin has been discovered. They are sually very pathogenic for man, chiefly in mixed and secondry infections as in blood poisons, streptococci sore hroat from milk infections, in pneumonia, consumption rheunatic fever and many others. The septic sore throat epiemics appeared in England in 1911, Baltimore and Chicago n 1912, and in many other cities. In 1917 a recurrent epiemic was common in many cities. Streptococcus is very

pathogenic for the lower animals. The prophylaxis consist in injections of immunized horse serum, which confers a immunity but not to such an extent as the immunity afte recovery from an infection. This immunity is very high.

The Pneumococcus or diplococcus pneumoniae is commo in acute inflammation of the lungs. Although a very smal organism, it has many names as Diplococcus or streptococcu pneumoniae, micrococcus lanceolatus, pneumococcus an Frankel's pneumococcus. It is a small elongated coccus; on end is lance shaped and commonly occurs in pairs. Shor chain formation is common when grown artificially. It i capsulate, staining with the common stains and is Gra positive and non-motile. Growing best in blood serum medi and in serum agar appearing as small moist, translucent well defined edged, granular colonies, surrounded by a green ish colored zone. Milk is acidified but not always coagulate The organism has been divided into four types or varieties See pages on pneumonia. The organism commonly inhabit the normal nose and throat, in the air and secretions from th nose and mouth. It is pathogenic for man and usually as sociated with other organisms. It also is pathogenic fo lower animals. The organism produces a toxin (not soluble) agglutinins and precipitins. Prophylaxis to some extent i acquired by immune serums and bacterial vaccines. An at tack of pneumonia will not produce an absolute immunity

Meningococcus or micrococcus meningitidis, inflammatio of the brain and spinal cord is produced by these and othe organisms, one form of which is epidemic meningitis, spotte or cerebrospinal fever. It was discovered by Leichtenster in 1885 and fully described by Weichselbaum in 1887. Th organism appears as a diplococcus or tetrad usually in th interior of the polymorphonuclear leukocytes. They are Gra negatives (differing from many other diplococcus). No cap sule has been found. They grow best on blood media and ap pear in solid blood media as elevated, smooth, moist, blu grey colonies. They are not green or hemolytic as the hemo lytic streptococcus or the streptococcus viridans. They ar not so whitish and opaque as the staphylococcus. They liv only a few days on blood media, but in starch agar for sever al weeks. Dextrose is fermented by meningococcus and gono coccus, and maltose by meningococcus; micrococcus catarrh alis produces no fermentation. Meningitis is acquired throug mouth sprays from infected persons. It is present in man normal persons, whose bodies may be weakened by insufficien

lothing, fatigue and exposure. These often produce the lisease. It is very pathogenic for men, especially children. The disease is rarely found in persons over 35 years of age. The lower animals are not very susceptible but may act as arriers. Prophylaxis is to some extent obtained by an immune serum. The action of this serum is said to be by agglutinins and antitoxic action.

The Gonococcus or micrococcus gonorrhea is one of the most widely distributed organisms. It was discovered by Neisser in 1879, who demonstrated its constant presence in gonorrheal pus. Pure cultures were obtained in 1885 by Bumm. In appearance it is similar to the meningococcus, always occurring in pairs with the flattened sides adjoining, and resembles a coffee bean. In pus it is found both inside and outside the leukocytes. The cocci do not invade the nucleus; and as the cocci are non-motile the phagocytic action is clearly seen. They stain with the ordinary stains and are Gram negative; but as they are slowly decolorized, the alcohol decolorization should be prolonged at least 10 minutes. They grow best on blood media. They are very pathogenic for man but not for the lower animals. No true toxin has been discovered. The organism is resistant to heat. See pages on Gonorrhea.

Micrococcus catarrhalis was found by Kircher, Pfeiffer and Sefert, in sputum and tissues of bronchitis, whooping cough, pneumonia and other respiratory diseases. It resembles the meningococcus and is Gram negative, growing best on blood media. It is pathogenic for man, but only partly so for lower animals. It does not ferment dextrose or maltose.

Micrococcus zymogenes was first seen by MacCallum and Hastings. It appears as a small micrococcus, mostly in pairs and sometimes in short chains. It grows readily on agar, producing acid in glucose or lactose and slowly liquefies gelatin. It produces acid and curdles milk, and the curd is slowly dissolved. It is pathogenic for lower animals, producing local abscesses or a general septicemia. It is pathogenic for man.

Micrococcus tetragenus was discovered by Gaffky in the lung cavities of consumptives, and in abscesses in men and animals. It is often found in healthy mouths. It appears in tetrads, or in groups of four small oval cocci, Gram positive; and grows on agar as a confluent elevated whitish rough colony. It will not curdle milk or liquify gelatin. It produces rapid septicemia in the lower animals and is pathogenic for man.

Anthrax Bacillus, causing anthrax or splenic fever i one of the oldest studied bacterial diseases. In 1850, Davine Pollender and Rayer found the rod shaped organism an proved Koch's laws. The bacillus is one of the largest patho genic organisms, usually occurring single, sometimes tw united end to end. It is capsulated; Gram positive, grow artificially—the bacillus may occur in filaments—and resem bles a jointed cane fishing rod or chain like formation. It i spore forming. One spore is produced in each bacillus, bein formed in the middle of the cell. On ordinary culture medi it grows aerobically; in broth no pellicle forms, but a sedi ment is produced. In petri cultures long wavy filament project from the colony in all directions. It slowly liquefie gelatin—produces no indol, curdles and acidifies milk. It i very pathogenic for lower animals—cattle, horses and pig On man it forms malignant pustules, or it may infect th lungs or intestines. Persons handling sheep, butchers, hide o hair handlers are liable to become infected. No toxin has bee isolated. Vaccination against anthrax is successful, by usin attenuated cultures, the first dose being a broth culture s weakened that it will not kill guinea pigs; and the secon dose, a broth culture which will kill guinea pigs but not rab bits. Kraus used for human anthrax, injections of norma beef serum which had been heated two times for one hal hour at 56 degrees C., in doses of 10 to 30 mils.

Bacillus Subtilis or the common hay bacillus, is simila to anthrax in the cultures and morphology. The spores for in the middle instead of at the end as in anthrax. No pel licle forms in broth cultures. Gelatin is liquefied rapidly The organism is found in the air, earth and water and is us ually non-pathogenic. The potato bacillus—Bacillus mesen tericus—is one of this group. Bacillus subtilis is easily isolat ed by chopping hay into small pieces, placing them in wate and boiling for one hour, killing practically all other organ isms; but the spores of the hay bacillus are so resistant tha when the hay infusion is inoculated into culture media, pure culture results.

Bacillus diphtheriae was found by Klebs in 1883 and th description completed by Loeffler in 1884. The organism is slender rod, and when stained appears as beaded, striated o granular. It has a tendency for involution forms, for i some cases branching by budding may be seen. It is usuall Gram positive. It grows best on Loeffler's serum (calf o

heep serum 3, glucose broth 1.) and appears as small opaque ray colonies, on the surface, in 12 to 18 hours. It will not quefy gelatin—growing readily in milk with a slight acid eaction. Cultures may retain their virulence for over one ear. Exposing to 55 degrees C, in moist heat 45 minutes ills the organism. In dry heat they may be viable after one our's exposure to 98 degrees C. It is found in the body sually on mucous surfaces on the throat, nose and eye, but ay occur in other places as in the ear. It produces a sol- ble toxin, when grown in broth. As a prophylaxis and cur- tive measures, isolation and the antitoxin are used. See ages on antitoxin. The disease is distributed by human car- ers (sometimes animals, as cats). In nearly all cases of iphtheria there is a mixed infection, streptococcus, staphy- coccus and other organisms. The diphtheroid bacillus close- resembles that of diphtheria but is less virulent and sel- om found in healthy noses and throats, where the diphth- ria bacillus is commonly found. It is somewhat shorter and ider and the granules are not seen. The diphtheroid bacilli ave been divided by Morse into four sub-groups:

FERMENTS	Dextrose.	Sucrose.	Maltose.	Gylcerin.
acillus hoagii (very common)	+	+	—	—
acillus flavidus	+	—	+	—
acillus xerosis (very rare)	+	—	—	+
acillus Hoffmanni	—	—	—	—

Mellon has added three more sub-groups: B. diphtheroids quificiens, B. enzymicus, which is closely related to the reptococcus, and B. Ruedigeri. The B. enzymicus, flavidis nd Ruedigeri are the most pathogenic. It has been found hat about 20% of the healthy people harbor the pseudo- iphtheria bacilli, and that 2% of all persons have the gran- lar type of diphtheria organism, and that 17% have viru- nt organisms. See pages on Diphtheria.

Bacillus Coli or the colon bacillus was discovered by scherich in 1886. These organisms are widely distributed n nature, in the air, soil and water. They are easily isolated rom fresh healthy feces, and also from many of the higher nimals. Those isolated from dog feces are more virulent for uinea pigs than from the intestines of rabbits or guinea igs. The bacillus varies in shape and size from that of a ery short oval form to the typical bacillus form. It will urdle milk but will not liquefy gelatin, producing gas in extrose and lactose. The most frequently found bacillus of he coli group are the B. neapolitans or B. coli communior hich produces gas in saccharose and dulcite, B. coli or B.

coli communis which produces gas in dulcite, B. aerogene which produces gas in saccharose, and B. acidi lactici whic with not produce gas in saccharose or dulcite. The coli grou of bacillus are pathogenic to man. Under certain condition they are able to pass from the intestines to other parts c the body and cause disease. Closely allied to this group ar the B. bifidus which is Gram positive and reproduced by a d vision at one or both ends; and B. acidophilus which is a anaerobe and is Gram positive. They are found in th intestinal tract of infants.

Bacillus aerogenes often found in sour milk, and some times called B. lactis aerogenes, resembles the coli bacillu in many ways, and many times associated together in th intestines—growing in gelatin, with the characteristic "na head" appearance, rapidly curdling milk and producing fe mentation. These organisms are non-motile and capsulate and not supposed to be pathogenic to man.

Bacillus pneumoniae or Friedlander's pneumobacillus, is capsulated pathogenic bacillus. It is Gram negative an grows on ordinary media. It is pathogenic for the lowe animals, producing suppurating lesions.

Bacillus capsulatus or B. mucosa is similar to the Baci lus pneumoniae and is found in a number of pathological cor ditions.

Bacillus ozenae is found in catarrhal conditions of th nose and throat and is a capsulated bacillus, usually produc ing no fermentation.

Bacillus enteritidis is similar to the Gartner's bacillu which is found in food poisoning, by persons eating spoile food. It is called Gartner's bacillus on account of bein discovered by Gartner in 1888.

Bacillus suipestifer is very motile and capsulated. I has from 3 to 9 flagella. It grows in ordinary media, produc ing gas but not coagulating milk.

Bacillus susepticus is one of the hemorrhagic septicemi group, and is non-motile, growing feebly in ordinary media producing acid in milk and dextrose, but no curdling or gas This organism and B. suipestifer are the cause of hog chol era.

Paratyphoid bacillus is divided into two classes, viz Type A and type B. Type B is more commonly found an will ferment xylose and dulcite, blacken lead acetate agar i 18 to 24 hours and is more pathogenic for lower animals, an resembles in man the typhoid bacillus. The type A produce

alkali in litmus milk slowly, usually in not less than 14 days, while type B will produce alkali in 4 to 5 days. Type A will not ferment xylose and dulcite and will not blacken lead acetate agar. There is also a difference in their agglutinations. Many cases of these infections come from meat and milk poisoning, and some come from infected water.

Bacillus psittacosis, one of the coil group, is very pathogenic to man.

Typhoid bacillus, or Bacillus typhosus was discovered by Eberth in 1880; it was first grown by Gaffky in 1884. It appears as a short wide rod shaped organism, very motile and is Gram negative and non-sporing, growing on gelatin as thin bluish-white, irregular, notched margin colonies. See pages on Typhoid fever. These organisms come from the human intestine only, and all Bacillus typhosus can be traced to the discharges from a typhoid patient or a carrier. The organisms are very pathogenic to man but lower animals are not affected, although they may act as carriers.

The Dysentery Bacillus, or Bacillus Dysenteriae was discovered by Shiga, in 1898. It appears as non-motile and in staining and growth resembles that of Bacillus Typhosus; but it produces an alkaline reaction in litmus milk and will not decolorize neutral red agar. Four types of Shiga's bacillus have been separated and they are differentiated as follows: Type 1, or Shiga's type, will produce acid in dextrose only, and no indol is produced; type 2, or Park and Hiss type, produces acid in mannite and dextrose only, and produces indol; type 3, Flexner, Strong and Manila type, produces acid in mannite, saccharose and dextrose, and produces indol; type 4, or Harris, Wollstein type, produces acid in mannite, maltose, saccharose and dextrose, and produces indol. The dysentery bacillus is pathogenic for man and less so for animals. An endotoxin has been obtained. The prophylaxis is a polyvalent immune serum which has been used with good results.

Bacillus fecalis alkaligenes, found in feces and water, resembles the typhoid bacillus, except that it has many polar bodies instead of the flagella, and produces alkali in milk ond mannite media, but no gas in glucose. It is slightly pathogenic for the lower animals.

The name Hemorrhagic septicemia applied by Hueppe in 1886, to a group of fatal infectious diseases of the lower animals, in which many scattered small hemorrhagic areas were found, the chief being:

Bacillus pestis is a short non-motile bacillus, with bipolar

bodies, Gram negative and non-sporing, appearing usually in pairs. It will not liquefy gelatin, or coagulate milk. This organism caused the disease of the middle ages known as "black death." In India and South America epidemics of this disease are common. It grows at 30 degrees C. on agar media as tiny drop like rounded granular uneven margin colonies. If the surface of liquid media is covered with oil after inoculation, and placed undisturbed in the incubator for 5 days, long thin filaments hanging from the top of the liquid are seen. The disease is carried by droplet infection, or contact, or by animal carries as rats. It is very pathogenic for man and also for many of the lower animals. As a prophylaxis and immunity bacterial vaccines are used. An immune serum has also been used.

Noguchi divides the spirochetes into six different genera—Spirochaeta, Saprospira, Cristispira, Spironema, Treponema, and Leptospira. Of these groups the first three are of no particular significance.

The first organism of the spirocheta (coiled hair) group was the first spirocheta to be described and was due to Ehrenberg in 1838. Several species of this genus have since been found in salt and fresh water and are characterized by their considerable length (100 to 500 micra), by the fact that they have no membrane, that they contain plasmic spirals and volutin granules.

The saprospira (putrid-coil) group were described first by Gross in 1911. They are also long forms, though not so long as the spirocheta, and have a chambered structure. They occur in sand.

The cristispira (crested-coil) group, also described by Gross (1910), is exemplified in the Cristispira balbiani, an inhabitant of the crystalline style of shell fish.

Numerous members of the next, or spironema genus are of pathogenic significance. They are small, (8 to 16 micra) twisted micro-organisms with a permanent spiral filament and include many organisms that are the causative agents of the different forms of recurrent fever in man, formerly referred to as the Spirochetes of Obermeyer, such as Spiromena recurrents, S. carteri, and S. duttoni.

Spironema (coil-thread) is the word used to designate the organisms more familiarly known as spirilla, while treponema (to turn-thread) is used for the generic name of organisms similar to the Treponema pallidum. The name was

preferred even by Schaudin himself, to the more usual designation of spirochete.

The spironema group is separated from the treponema group. The treponemata as first described by Schaudin (1905) in connection with the causative agent of syphilis are somewhat shorter (6 to 14 micra) than the spironema organisms, and are characterized by pointed ends, a spiral filament and regular and rigid spirals. The causative agent of syphilis (Treponema pallidum) and of yaws (Tr. pertenue) are placed in this group; also non-pathogenic members of the group, like Tr. microdentium and Tr. macrodentium.

Leptospira (fine-coil), differentiated by Noguchi (1917). These organisms have pointed and hooked ends, a corkscrew-like appearance, and differ from other spirochetes in that they are very resistant to the action of saponin. Their locomotion, which is extremely active, differs somewhat in nature from the other varieties. The most important members of this genus that have hitherto been described are the leptospira of ichtero-hemorrhagic fever (L. icterohemorrhagiae) and the recently described Leptospira icteroides which is regarded by Noguchi as a causative agent in yellow fever.

Aniline gentian violet. Must be made as used. Saturated alcoholic solution of gentian violet 1. Aniline oil water 3. Mix and filter.

Aniline oil water. 2 mils aniline oil mixed with 100 mils water, shake 5 minutes and filter twice.

Antiformin. Equal parts of 7.5% sodium hydroxide and sodium hypochlorite (5.3 grams Cl. to 100 grams water).

Antiformin stain for the Bacillus tuberculosis.—Equal parts of antiformin and sputum are boiled until a clear, pale brownish liquid results. This will destroy all but the acid fast organisms. Cool this liquid and to 10 mils, add 1.5 mils of the mixture of chloroform 1. and alcohol 9. Shake to emulsify and centrifuge for 15 minutes. Between the layer of chloroform and alcohol and in the supernatant liquid a disk is formed which contains the bacteria. This disk is withdrawn from the centrifuge tube and is mixed with fresh egg albumen on slides and stained by the acid fast stain—10 mils of a saturated alcoholic solution of basic fuchsin and 100 mils of a 5% solution of phenol. It is then decolorized with alcohol containing 5% hydrochloric acid, washed in water and counter stained with Loeffler's methylene blue solution.

Antiformin solution. 100 mils of sodium hypochlorite containing 5.68 grams of available chlorine, sodium hydroxide 7.8 grams, sodium carbonate 0.32 grams. The chlorinated lime is dissolved in water at 35 degrees C.; to this is added the solution of sodium carbonate; after settling, the supernatant liquid is decanted and to this is added the sodium hydroxide. This solution is said to dissolve all cells and bacteria except the resistant acid fast bacteria.

Blood Stains

Eosin 0.1 gram in 1 mil of alcohol and 1 mil of water. Fix the filmed slide with heat, stain 30 seconds, wash with water, and counter stain 30 seconds to 60 seconds with an aqueous saturated methylene blue solution; or

Yellowish eosin 1 gram dissolved in 100 mils of methyl alcohol; stain the fixed filmed slide for 60 seconds; drain without washing; and then stain with methylene blue (1 gram in methyl alcohol 100 mils) for 60 seconds; wash with water; or

Flood filmed slide with methyl alcohol; immediately set fire to the alcohol; when cool stain with haematoxylon solution 312 mils and eosin 1 gram for 15 seconds; wash with water; or

One-half percent alcohol solution of eosin; stain 30 to 60 seconds; wash with water; stain with Loeffler's methylene blue 1 to 3 minutes; wash with water; or

Harlan's stain. Stain 20 seconds with an aqueous solution of eosin 0.5 mil and methyl alcohol 50 mils. This solution must be kept tightly corked. Drain slide without washing; then stain with methylene blue 0.5 grams and methyl alcohol 50 mils, for 20 seconds. Wash with water. If there is no blue in the stain add 1 drop of 20% potassium hydroxide solution; if too blue add 1 drop glacial acetic acid. Too much blue means the stain is too alkaline; too much red, the stain is too acid.

Eosinate of methylene blue. Add to 95 mils of methyl alcohol, 5 mils of tenth normal sodium hydroxide solution; shake; then add water soluble yellowish eosin 0.47 grams, and methylene blue 0.47 grams. Shake for 30 minutes and filter. In staining, flood the filmed slide with this stain for 60 seconds; at the exact end of this time, drop on the slide twice as much water as stain remaining on the slide; the correct amount of water can be told by the metallic scum which will occur. After counter staining with the water for 120 seconds, wash in water and examine. If the leukocytes are not sufficiently blue, add a drop of alkali; if the erythrocytes are not sufficiently red add a drop of acid to the stain.

McNeal's Stain. Consists of two solutions. No. 1 containing a 0.1% solution of water soluble yellowish eosin in methyl alcohol. No. 2, Methylene blue 1 gram, methylene azure 0.20 gram, methylene violet 0.60 gram and methyl alcohol 500 mils. This stain is used in the same way as the eosinate of methylene blue.

Hemin crystals. To a large drop of fresh blood on a clean slide add 20 drops of glacial acetic acid; evaporate in the air; then add 1 drop each sodium chloride solution and glacial acetic acid, warm gently and the characteristic brownish red crystals will appear.

Diluting fluid for blood counting. Toisson's fluid, sodium chloride 1 gram, sodium sulphate 8 grams, glycerin 6 mils, water q. s. 100 mils. Haymen's solution, mercuric chloride 0.5 grams, sodium sulphate 5 grams, sodium chloride 1 gram, distilled water 200 mils. For counting the white blood cells the diluting fluid is a 1.5% solution of acetic acid—this destroys the red blood cells, which makes the counting more easy. We prefer to add to the diluting fluids for red blood cells, sufficient saturated alcoholic solution of gentian violet

to tinge the solution and for the white blood cells, sufficient saturated alcoholic methylene blue solution to tinge the fluid blue; this will stain the blood cells and make counting more easy.

If one half of a blood filmed slide is covered with another slide and held tightly between the fingers so that all air will be excluded from the film, then the exposed film breathed upon several times, the red blood cells will disappear, leaving only the white blood cells. When the slide is stained, one half will contain the red cells and white cells, the exposed half will contain only the white blood cells.

Staining body fluids and their sediments. The specimen is placed on a glass slide, air dried and a 0.20% solution of eosin in absolute alcohol is placed on the specimen for several seconds; it is then drained off without washing but by tilting the slide; when the slide is dry, a drop of a mixture of neutral glycerin 2 parts and Unna's polychrome methylene blue 8 parts is added. A cover glass is placed over the specimen so that the stain is spread evenly and the excess squeezed out and blotted; it is then ready for examination.

Vibert's Solution for testing blood stains. The scrapings from the area as in the case of clubs, earth and knives or a portion of stained cloth is placed on a slide and soaked in a drop of Vibert's solution which consists of 0.5% mercuric chloride in a 2% solution of sodium chloride; or in a drop of glycerin 1 part to 7 parts of water. A cover glass is placed over the specimen and the slide after standing for 30 minutes is examined for the mammalian blood cells and the oval, granular nuclei of the non-mammalian blood cells.

BACTERIAL STAINS

Carbol fuchsin. Saturated alcoholic solution of fuchsin 10 mils and phenol 5 mils.

Carbol fuchsin stain for the cold method. Basic carbol fuchsin 4 mils, phenol crystals 8 grams, alcohol 20 mils. The slide is stained for 10 to 60 or more minutes and decolorized with 10% hydrochloric acid.

Czaplewski's carbol fuchsin stain. Fuchsin 1 gram, phenol 5 grams, glycerin 50 mils; this is mixed, then 100 mils of water are added. The stain is added to the fixed film on the slide, washed off at once and counter stained with Loeffler's methylene blue for 15 to 30 seconds, washed in water and examined.

Gabbet stain for the combined staining and decolorizing acid fast bacilli, is used as follows: The specimens are stained with hot carbol fuchsin as usual, the carbol fuchsin is drained off and the mixture of methylene blue 2 grams in 100 mils of a 25% sulphuric acid is placed upon the slide for a short time; wash the slide in water and examine.

Pappenheim's acid fast stain. A 1% solution of corallin or rosolic acid in absolute alcohol and saturated with methylene blue, then add 20% glycerin.

Carbol thionin. Saturated solution of thionin in 50% alcohol, 10 mils, 2% phenol 100 mils. Stain for 2 minutes.

Smith's formal fuchsin. Saturated alcoholic solution of fuchsin 10 mils, methyl alcohol 10 mils, formalin 10 mils and distilled water q. s. 100 mils.

Orth's acid alcohol. Hydrochloric acid 1 mil, and 70% alcohol 99 mils.

Carbol-gentian violet Gram stain. Saturated alcoholic solution of gentian violet 90 mils and 5% phenol in water 1000 mils. This solution retains its staining powers for a longer time than the ordinary aniline gentian violet solution, but is not so permanent as the Sterling modification.

Sterling's modification of the Gram method, 2 mils of aniline oil and 10 mils of 95% alcohol are shaken together and 88 mils distilled water added. This solution is slowly added to 5 grams of gentian violet in a mortar and ground. This solution keeps well and stains in 30 seconds. After being ground and mixed, it is shaken for 24 hours and filtered before using.

Modified Gram. The slide is flooded for 10 seconds with 1% crystal violet solution (saturated alcoholic solution of crystal violet 10 mils, 15% aqueous phenol solution 90 mils); then add without washing for 10 seconds, or until black. Gram's iodine, then wash with 95% alcohol for 10 seconds, wash quickly with water and counter stain with a 1% aqueous solution of eosin. Gram positive are blue-black, Gram negative are reddish.

Gram's Iodine. Iodine 1 gram, potassium iodide 2 grams, distilled water 300 mils.

Or stain with 40% formalin 5 mils and 1 mil of a saturated alcoholic solution of gentian violet 1 part with water 95 parts, wash in alcohol, stain with the Gram solution and counter stain with Loeffler's methylene blue.

Gram positive bacteria are killed by a 1 in 1000 solution of gentian violet but the Gram negative are not killed.

CAPSULE STAIN. A thin film is stained for 1 to 2 minutes with carbol fuchsin. It is then washed in alcohol, then washed thoroughly with water. Or stained for 10 minutes with a mixture of saturated aqueous solution of mercuric chloride 2 parts, tannic acid 20% aqueous solution 2 parts, saturated aqueous solution of potash alum 5 parts. Then 95% alcohol is added for 1 minute; the preparation should now have a pale rose color. Wash well with water. Counter stain with methylene blue 1 to 2 minutes. Dehydrate in alcohol for 5 minutes, dry and mount in balsam.

The Hiss copper sulphate method of capsule staining. Cover slip preparations are made by mixing the organisms with a drop of serum (beef blood serum). Dry in the air and fix by heat. Stain for a few seconds with a saturated alcoholic solution of fuchsin or gentian violet 15 mils in 85 mils of distilled water. Hold the cover glass over the flame until it steams; wash off the stain with a 20% aqueous solution of coper sulphate. Blot—do not wash—dry and mount.

Differentiating bacteria killed from heat or disinfectants from those which are living or killed by natural causes.

The bacteria are placed on the slide and fixed in the usual way. They are then stained with concentrated carbol fuchsin 8 mils and Loeffler's methylene blue 10 mils for 24 hours. The dead bacteria stain red, the living blue. Or potassium tellurite in dilutions of 1 in 50,000 forms the characteristic black compounds in the living bacteria, but no reaction with the dead. This reaction should be carried out at 37 degrees C. for 16 to 90 hours.

STAINS FOR THE BACILLUS DIPTHERIAE.

Neisser's stain. Solution No. 1 composed of methylene blue 1 gram, alcohol 200 mils, glacial acetic acid 50 mils and distilled water 1000 mils, is added to the heat fixed slide for 30 seconds. It is then washed with water and stained with solution No. 2, composed of bismark brown 0.2 grams and boiling water 100 mils, for 30 seconds, washed in water and examined.

Or the fixed slide is stained by steaming once over the flame with glacial acetic acid 5 mils, old saturated alcoholic solution of methylene blue 4 mils, distilled water 95 mils, alcohol 100 mils, and carbol fuchsin 4 mils, well mixed and filtered. As soon as the stain on the slide begins to steam, the stain is set on fire and the alcohol burned off; the slide is cooled and washed well with water. The bacilli stain red, the polar bodies a deep blue.

Roux stain. Dahlia or gentian violet 0.5 grams, methyl green 1.5 grams, dissolved in 200 mils of distilled water; the slide is stained 5 to 10 seconds, washed in water and mounted. Tissues are stained by this stain by staining for 12 hours.

Staining for 3 minutes in carbol fuchsin, decolorizing with 25% nitric acid, washing with alcohol and standing for 3 minutes in formaldehyde, and washing in water, stains the bacillus a dark violet.

Toluidin Blue mixture for diptheria bacilli. The stain toluidin blue 0.1 gram, glacial acetic acid 0.5 mils and distilled water 100 mils, is placed over the air dried slide; a cover glass is placed on the stain; the excess blotted and the slide examined. The bacilli are faint blue, while the polar bodies are a deep reddish color.

Loeffler's stain or Loeffler's methylene blue, or Loeffler's Alkaline Methylene Blue, 30 mils of a saturated alcoholic solution of methylene blue and 100 mils of a 1 in 10,000 solution of potassium hydroxide in water.

Stitt's Diptheria Stain. The filmed slide is stained for 2 minutes with a saturated alcoholic solution of gentian violet 25 mils and 75 mils of a 5% solution of formaldehyde; at the end of this time the slide is flooded with ½% Gram's Iodine for 1½ minutes, thoroughly washed in 95% alcohol and counterstained with a saturated aqueous solution of bismark brown for 4 minutes. The bacilli stain dark brown and the granules a deep violet.

FLAGELLA STAIN. Flagella are best seen in 18 hour cultures and on account of the flagella being delicate, care must be exercised in spreading on the slide and the slide dried at 37 degrees C. It is best to carefully inoculate a tube of sterile water with sufficient of the culture to produce turbidity; after the tube stands for a little time, the organisms fall to the bottom and are easily transferred to the slide without an attempt to spread the organisms on the slide.

Loeffler's method. The slide is kept warm over the water bath with a 25% aqueous solution of tannic acid 10 parts, saturated aqueous solution of ferric sulphate 5 parts and saturated alcoholic solution of fuchsin 1 part for 5 minutes, care being taken that the slide is always covered with this solution which is the mordant. Wash well with water and dry with blotters. Cover the slide with aniline gentian violet or carbolfuchsin and heat over the water bath for 5 minutes, wash well with water and mount.

Van Ermengen's method. The slides are placed in osmic

acid 2%, 1 part and 25% tannic acid 2 parts for 1 hour or may be heated for 5 minutes over the water bath. Wash with water, then with absolute alcohol, and again with water; then cover with a 0.5% aqueous solution of silver nitrate for 20 seconds; then transfer to the solution of gallic acid 5 grams, tannin 3 grams, fused potassium acetate 10 grams and distilled water 350 mils, for 30 seconds; then treat with the silver nitrate solution until the film turns black, wash and examine.

FONTANA'S SPIROCHAETA STAIN. The material from the syphilitic lesion is spread very thin on a slide and air dried. Fix for several seconds with this solution, acetic acid 1 mil, formaldehyde 20 mils, distilled water 100 mils, pouring off this solution and repeating several times. This solution during the repeated fixings should be on the slide at least one minute. Wash in distilled water. Flood with this mordant, tannic acid 5 grams and 1% phenol solution 100 mils, and warm until steam arises; continue this process for at least 30 seconds, wash in distilled water for 30 seconds; then flood with a 0.25% silver nitrate solution, for 30 seconds, wash with distilled water; dry and mount. The spirochetes are stained a jet black.

Or stain for 10 minutes with this solution: Phenol crystals 50 grams, tannin 4 grams, water 100 mils, mix and add 2.5 grams fuchsin and then 100 mils absolute alcohol. Wash in water, dry and counter stain with an aqueous solution of gentian violet 10 mils, concentrated aqueous solution of phenol 10 mils and water 100 mils for 15 minutes; wash in water, dry and mount.

Or stain the air dried slide for one minute in acetic acid 1 mil, formalin 20 mils, distilled water 100 mils; wash in water; then add phenol 1 gram, tannic acid 5 grams and a 25% silver nitrate solution 5 mils and steam for 30 seconds; wash in water than add ammonia water and steam for 30 seconds; wash in water, dry and mount.

SPORE STAIN. The spores stain deep red and the bacteria blue with the following stain; the slide is covered and steamed for 1 minute with acid fuchsin or eosin 4 grams 2% acetic acid 50 mils and methylene blue 2 grams; it is then washed in water and then stained with a saturated solution of sodium bicarbonate for 30 seconds, washed with water, dried and mounted.

Or air dried slides are fixed in a saturated aqueous solution of mercuric chloride for 30 seconds; washed in water

and stained for 30 seconds with methylene blue 1 gram, sodium carbonate 1 gram and water 100 mils; washed with water, dried and mounted.

URINE STAIN. To the sediments from the centrifuge tubes add a 0.4% sodium chloride solution; shake well and drain; repeat this process 3 times. Transfer a platinum loop full of the sediment to a slide, air dry, then add a 5% aqueous solution of mercuric chloride for 3 minutes, wash with water and stain with 1 mil of a 0.3% aqueous solution of methylene blue and 1 mil of a 0.02% aqueous solution of fuchsin for 3 minutes. Wash in water, dry and mount. Casts and protoplasm are pink to dark red, waxy casts are bright red, fat globules are bright orange, granular casts are reddish, mucin and chromatin violet to indigo and the protoplasm of the renal epithelial cells light pink.

SOLUTION FOR CLEANING SLIDES. All slides and cover glasses for blood and bacteriological examinations must be cleaned with this solution: Potassium dichromate 80 grams, water 300 mils, heat and cool; then carefully add commercial sulphuric acid 460 mils; place slides and covers in this solution for 12 hours. Wash in water dry with lintless cloth and preserve in alcohol.

Stock Staining Solutions.

Saturated solutions of

Fuchsin in alcohol.....3%
Gentian violet in water.....1.5%
Gentian violet in alcohol.....4.8%
Methylene blue in water.....6.7%
Methylene blue in alcohol.....7%

Consult your U. S. P. for other stain formulas.

Add the formulas of other stains you may use.

PREVENTION OF DISEASE IN WAR

Of the 10,000,000 men called, 6,750,000 were not given medical examination. One third or 3,208,448, were examined, 70% were qualified, and 30% were partly or totally disqualified. Of the 2,1124,293 who were sent to the camps and there subjected to minute examinations 92% were accepted. In the draft of 1917, 29.1% were rejected; while in 1918 29.6% were rejected.

Typhoid fever started as an epidemic in Belgium in 1914 and spread with its old time fury among the troops, but was conquered by sanitation and vaccination.

Typhus fever spread in Serbia, Austria and Russia; but through the knowledge that it was carried by body lice, the epidemics were controlled.

Cholera started and was controlled in Russia and Italy.

Small pox appearing from recruits from civil life, has been so quickly controlled that there were from January, 1917, to April, 1919, only six deaths in the American army of over 2,000,000.

Emperor Frederick Barbarossa, in the middle ages, saw one army in Italy annihilated by sunstroke; ten years later, after conquering the city of Rome, pestilence swept away another army. In the thirty year war, the Swedish army fought its way from the Baltic to Vienna, where the bubonic plague worked such havoc with the forces that they were forced to withdraw and lost the campaign, so brilliantly won. In the middle of the eighteenth century, the bubonic plague again raged; this time among the Austrian and Russian armies, forcing these armies to bring the war to an unexpected end and make unfavorable peace with Turkey. In the thirty year war between the Protestant Swedes and northern Germans, aided towards the end by the French, against the imperial Catholic armies of Spain, Bavaria and Austria in 1618-1648, small pox was ever present; typhus, dysentery and scurvy added their toll to death; and after 1832 the bubonic

plague imposed its terrors. Wurtemburg during five years by war, famine and pestilence lost over 300,000 persons.

In the Electorate of Saxony, bubonic plague, typhus and dysentery in two years carried off 934,000; three quarters of the entire inhabitants of Germany, over whose fields the war had been waged, were blotted out of existence. Typhus still raged during the wars of the Spanish succession and in the Seven Year War of Frederick the Great. During all this time efforts were directed to cure and not to prevent. In the Napoleon war against Prussia in 1806-1807, typhoid fever was recognized and separated from typhus, which ravaged both armies and population. The retreat from Moscow and the Russian campaign of 1812, was probably the greatest military disaster of modern times; for out of the 680,000 who crossed the frontier with Napoleon, 80,000 men were down at one time with dysentary, and of the remaining soldiers, 100,000 were killed in battle, the balance perished from cold, disease and starvation. The pursuing Russians in three months lost 62,000 men from typhus. In the German campaign a year later, the army lay scattered amid villages on the retreat from Germany, the men dying by the thousands with a disease rate of 141. In the German army, the ruin of the army being due to neglect of hygiene., Following the Russian campaign 3,000,000 contracted typhus, spread broadcast by Napoleon's scattered armies. The Spanish war from 1808 to 1814 cost France 90,000 killed, and deaths from disease over 460,000. First there ravaged typhus, later in 1810 and 1811, yellow fever. In Saragossa of the 100,000 inhabitants 54,000 died of typhus, and of the 30,000 soldiers killed, 18,000 died of typhus. In the English peninsular campaign during 1808 and 1811 when the English attempted to take Antwerp from Napoleon, the ill-fated expedition of 42,000 men, lost 206 men killed from wounds and over 8,000 through disease. During this campaign, typhus fever was somewhat controlled, but dysentery and typhoid caused 11,000 out of the 14,000 deaths from disease.

In the American Army in the Mexican war, 1846-1848, seven times as many men died from disease as from battle wounds, or a mortality of 110 per thousand, while the battle loss was fifteen per thousand. The Crimean war, 1854-1856 shows the highest loss from battle casualties among the Russians, and from disease by the French. Cholera was carried by the French soldiers causing 12,457 cases, among 262,000 men and the English army had 4,531 cases. The battle death rate among the British was 69 per thousand per year, among

the French 70, and among the Russians 120. The disease rate was 230 per thousand among the English, 341 among the French and 263 among the Russians. The English and French armies suffered two epidemics from Asiatic cholera, costing the English 4,513 and the French 10,044; in the first eight months of war, the deaths exclusive of wounds and cholera were 9,762 in the English army and 5,523 in the French. The English began hygienic sanitation and the death rate immediately dropped from May to August 1885 to 923 but the French not practicing these measures, the death rate rose to 10,545; from September to December 1855 the English death rate was 463 and the French 8,473. In the last four months, the British lost 218, while the French 17,129.

In the Seven Weeks War between Prussia and Austria in 1866, the Red Cross societies under the Geneva Convention of 1864, for the first time acted.

In the Franco-Prussian war of 1870, the Prussians reached the highest standard of hygienic protection; their battle casualties were fifty-five per thousand, to a death rate from disease of twenty-five per thousand. The French, however, were the opposite, the casualties were sixty-eight per thousand spreading a pestilence among the inhabitants, the the total losses were 28,500, of whom 12,000 died from disease, out of a total army of over 725,000. Three infectious diseases spread in this war: Small pox, typhoid and dysentery, and for the first time in the history of a large European war typhus did not break out. In the Prussian army, small pox occurred in 6 per thousand, in an army that was supposed to be vaccinated; typhoid ninety-three per thousand, and dysentery forty-nine per thousand. Among the French prisoners of war, small pox broke out as a plague, about 14,000 cases occurring in Germany and 25,000 cases in the interned army in Belgium. There were fifty-four per thousand among the prisoners in Germany, which was nine times that in the German army. This shows the difference between the unvaccinated and the vaccinated army. Up to this time in Germany, the population was supposed to be vaccinated; but without compulsory health laws, many neglected to be vaccinated, and small pox as an epidemic followed, causing the death of 170,000 persons after the war.

That epidemics have followed wars caused by the soldiers spreading the diseases is shown by the Franco-Prussian war when the French prisoners coming from infected districts spread and caused 170,000 deaths from small pox, this led in 1874 to compulsory vaccination laws. From Ger-

many small pox spread to Austria and Bohemia. In Vienna it increased from 7.6 per 1,000 in 1871 to 52.7 in 1872 and in Prague 1.5 per 1,000 in 1870 to 39.7 in 1872. Up until 1870 small pox was practically unknown in Germany. It was carried to England by refugees in 1870 and the death rate rose from 0.7 per 1,000 to 10.1 in 1871.

In our Civil War, the death rate from wounds was thirty-three per thousand, and from disease sixty-five. In the Spanish War the wound death rate was five, from diseases 30.4 per thousand.

During the Civil War, Connecticut had an epidemic of typhoid, dysentery and small pox, and in 1864 over 10,000 persons had these diseases in Massachusetts.

In the late war up to March 28, 1919, the death rate from wounds was 14.191 and from disease 14.797 per thousand.

In the American Expeditionary Forces with an average strength of 975,716, a wound death rate of 31.256 and from disease 11.283 per thousand. Of those who died from disease, pneumonia claimed 9.146 per thousand.

Malaria was one of the chief diseases in the former American armies, causing six per cent of the deaths in the Civil War and ten per cent in the Spanish American War; while in the recent war there was practically no malaria. Typhomalaria caused 22.4% of the deaths in the Civil War and 60.5% of the deaths in the Spanish American War, being one of the uncontrollable diseases.

During the Spanish American War, there were 1,346 cases of typhoid in Butler, Pa. (1903) and 1,350 persons in Ithaca, N. Y., had typhoid. Epidemics of typhoid appeared in Augusta, Me., in the same year and in 1903 and 1904 in Cleveland and Columbus; Lowell, Mass., in 1903, and New Haven, Conn., in 1901.

In the recent war it caused only 0.4% of the deaths.

Pneumonia caused 13% of the deaths during the four years of the Civil War; 3% in the five months of the Spanish American War; and in the recent war 85% of the deaths from disease. Meningitis in the Civil and Spanish American Wars caused 2% of the deaths; in the late war 4%. Small pox caused 4% of the deaths in the Civil War; one death in the Spanish American War and in the late war, one man died in the United States and five in France. Dysentery caused 28% of the deaths in the Civil War and nearly 30% of the 5,600,000 cases of diseases reported in that war. 5.6% of the Span-

shi American War, but only 41 deaths out of 48,000 or 0.08% of the deaths in the recent war. Yellow fever caused 1,300 cases in the Civil War, 1,100 in the Spanish American War and practically none in the late war. Pneumonia in the late war caused 85% of the deaths from disease, while in the Spanish American War, typhoid, a preventable disease, caused 60.5% of the deaths from disease.

In the Russian-Japanese war of 1894 and 95, strict examination and quarantine prevented epidemics. 94.3% of the army was effective for duty at all times, and of the 5.7% on the non-effective list, 3.4% were so rendered by disease. Of the 195,000 Americans wounded, the lives of 182,000 were saved; and 22,205 have died of disease while 55,518 died of wounds or were killed. The deaths from pneumonia were over 8,000; venereal diseases averaged 40 per thousand.

Statistics show that annually 875,000 men and women are disabled for more than four weeks as the result of accidents sustained in industry; that annually 76,000 people suffer loss of members, and at least 200,000 are otherwise permanently disabled by these industrial accidents, and that 28,000 of our people are annually killed by industrial accidents.

Because of the nonreporting of occupational diseases, it is impossible to obtain statistics; but it is conservatively estimated that at least 250,000 more people are annually permanently disabled as the result of occupational diseases, and the deaths from these reach into the tens of thousands.

MOLDS (HYPOMYCETES)

A subdivision of the Algae and belong to the higher Hypomycetes, chiefly to the subdivision Ascomycetes. They are usually chlorophylless, hence they are unable to supply their own food and are generally parasitic, or saprophytic. They consist of slender colorless filaments, ramifying among decayed organisms, or living cells, thus deriving nourishment from either plant or animals. They have two sets of organs; the "vegetative," which supply food; these are the "hypha," single simple filaments, and "mycelia," tangled masses of "hypha." The "reproductive" organs bear spore heads which are usually thrust above the "host," the substance from which they derive their nourishment.

Molds may be harmless, or cell destroying—pathogenic.

Out of the hundreds of species, the most common mold is the Penicillium glaucum, the common green mold, found on bread, jelly, etc. Mold is easily cultivated by placing a piece of moistened bread under a tumbler for 24 hours.

Molds are true fungi, closely related to bacteria and often found as contaminations in culture media, making it hard to differentiate the bacteria and molds. They are often the cause of the spoiling of food.

Some of the most common molds are:

Penicillium glaucum.—Living on widely distributed hosts. The hypha and mycelia divide into branches, or basidia from which tiny tufts or filaments arise—the sterigmata, which are arranged like a tuft or brush. On each sterigma a conidium forms, which contains the spores.

Penicillium italicum is the species which forms the green molds. The hypha are separate and branch dichotomously. The fruit head resembles a brush.

Penicillium crustaceum. The conidiophores have ventricillate branches and the sterigmata forms chains of conidia.

Mucor mucedo is a very common mold, found on horse-dung, nuts, fruits, bread, et al. It grows as a whitish mold, sending out branches, on a pointed stem. It forms the enlarged globular headed spore sac—the sporangium, which contains a number of cells.

Rhizopus nigricans is the black mold. The sporangium is a light brown color, and contains the sporangiphores—the spores, which are liberated by the bursting of the sporangium. If the sporangium is old it is almost white in color.

Aspergillus fumigatus. The spores are borne on the upper surface of a rounded sporangium. It is pathogenic to

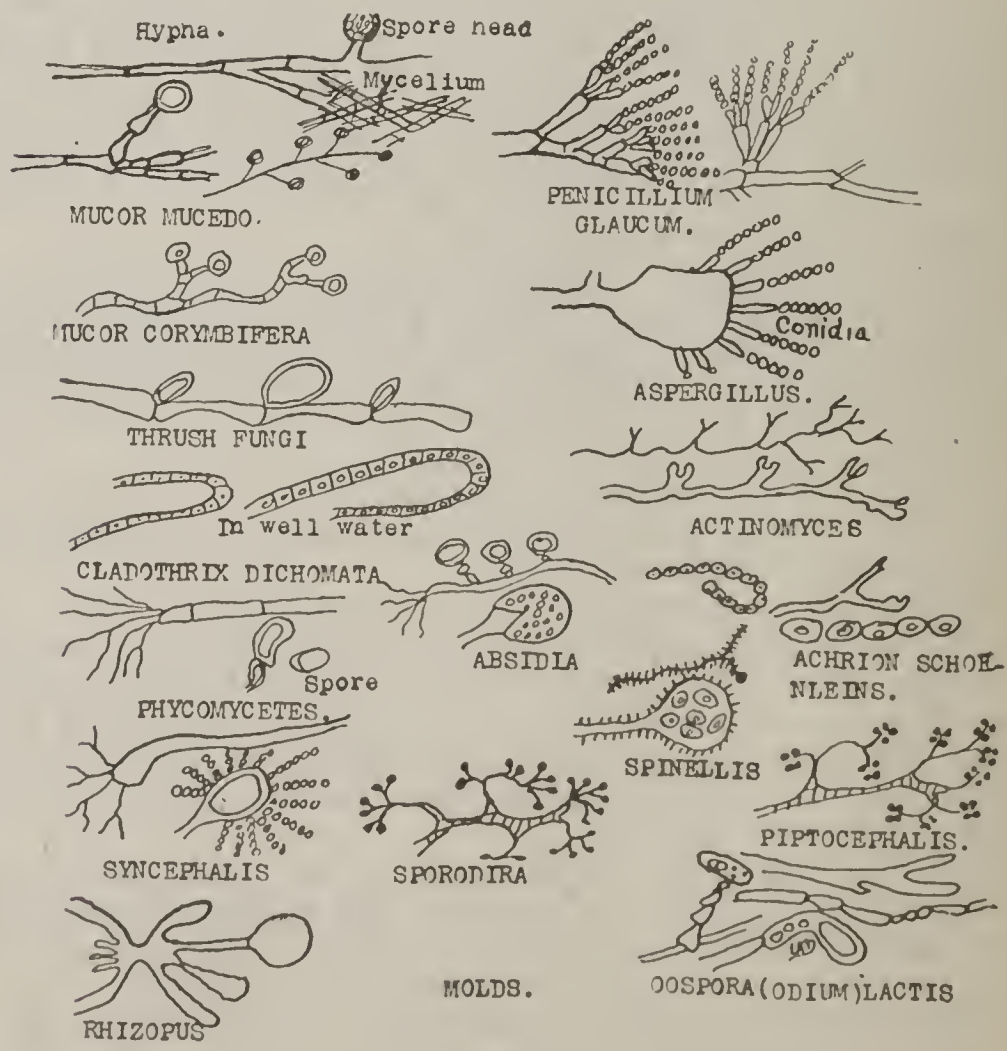
man and lower animals, infecting usually the lungs.

Aspergillus glaucus is found on saccharine fruits. One end of the hypha is enlarged into a pear shaped sterigma, which bears the conidia. This is one of the common green molds.

Aspergillus niger. Branches dichotomously, reproducing asexually, and the conidia form in chains from the enlarged headed conidiophore. The spores are black.

Aspergillus flavus reproduces by tuft like hyphae containing yellow spores.

Ospora-Odium lactis.—A white mold, which branches dichotomously. The hyphae are almost entirely submerged in the nutrient substrate-host. There are no typical fruiting



bodies. Reproducing by conidia formed by the simple division of the hyphae. The conidia are colorless.

Detection of mold in food products. As all fungi contain chitin, by boiling the suspected sample for one hour with a 50% sodium hydroxide solution, washing free from the alkali and treating with the reagent composed of Iodine 2 grams, potassium iodide 1 gram and water 2000 mils, then introducing the suspected treated material into sulphuric acid a violet color is produced if chitin is present.

EXERCISE

1. Rub a small portion of the colony with a mixture of alcohol 1 drop and ammonia water 1 drop. Then add 1 drop of glycerin (not glycerin jelly), cover and examine, and draw.

2. To another small portion of the colony on a slide, add 2 drops of a 10% KOH solution; gently warm; examine, and draw.

3. Treat another small portion with 1 drop of ether; then with 1 drop of alcohol; then 1 drop of water; then with 1 drop of 10% KOH solution, stand one hour then add 1 drop of glycerin and examine.

4. Stain another small portion with Loeffler's stain for three minutes; carefully wash with water; draw and examine; if good, make a permanent mount. Mount all molds, yeasts, bacteria and blood with canada balsam. Have the slide and cover glass absolutely dry; then place a small drop of the balsam on the center of the cover glass; invert it over the specimen and clamp. You must use sufficient balsam to fill the space between the cover glass and slide. If there is an excess, it may be removed after it has hardened, by chloroform.

5. Stain another portion by the Gram stain, make a permanent mount; draw.

SACCHAROMYCETES (BLASTOMYCETES)

The saccharomycetes, or yeasts, are true sac fungi. The cells are unicellular, globular, thin walled (cellulose) enclosing protoplasm containing one very large nucleus and many vacuoles. Containing no chlorophyll, they are unable to make their own food. Some yeasts are pathogenic and cause disease; but nearly all are non-pathogenic. They are divided into true and false yeasts. The true yeasts cause fermentation, such as produced in bread making, decomposing sugars or starches into alcohol, acetic and other acids and carbon dioxide gas. This gas causes the rising of bread. The gas being distributed throughout the dough and on account of the sticky nature of the dough, caused by "gluten" in the flour, cannot escape, thus causing the raising or puffing of the dough, which when in baking becomes porous caused by the escape of the gas, the alcohol is also lost by baking.

In brewing the "top" yeast is employed in making ale. Top yeast is the yeast which during activity raises to the top of the container. Ale, stout and porter fermentation takes place at ordinary temperatures and produces carbon dioxide gas so rapidly that the yeast is forced to the top of the vats. "Bottom" yeast grows at the bottom of the containers and is used in making lager beer.

Yeast cakes are yeast mixed usually with potato starch, the starch being added to absorb water from the yeast mass and to render it semi-solid, so it may be moulded into cakes.

The yeast cells reproduce by "budding," or by endospores. In budding, small protuberances appear on one cell wall (parent cell), increasing in size until about the size of the parent cell, and eventually becoming detached, (daughter cell), usually producing a new cell before becoming detached, so that they appear as a string of cells.

Cerevisiae Fermentum Compressum is the title in the N. F. given to the yeast cake composed of Saccharomyces cerevisiae or other species of Saccharomyces, Family Saccharomyetaceae. It is described as being of a starchy or absorbent base containing the most living yeast cells. Yeast cakes occur in compressed masses, or cakes and when viewed under the microscope the starch and yeast cells are seen. When stained with iodine T. S. the starch stains blue and the yeast light yellow. The yeast cells are oval or spherical, thin walled. The vacuoles contain small fat globules and the protoplasm is granular.

Some of the more common yeasts are:

Saccharomyces cerevisiae, the beer making yeast which is rounded or oval, and contains a granular mass enclosed by a thin membrane. Different kinds of beer are produced by the various species.

Saccharomyces rosaeus or Torula rosa and Saccharomyces niger and Saccharomyces albicans (which produces thrush) do not produce alcoholic fermentation but are pigment producers. They are found in the air.

Saccharomyces mycoderma is found on the surface of wines (called flowers of wine), vinegar and sauerkraut fermentations. It will not produce alcoholic fermentation, and is sometimes found on the human skin where it is pathogenic.

Saccharomyces apicutatus, or ellipsoideus, one of the wild yeasts which is necessary in wine making.

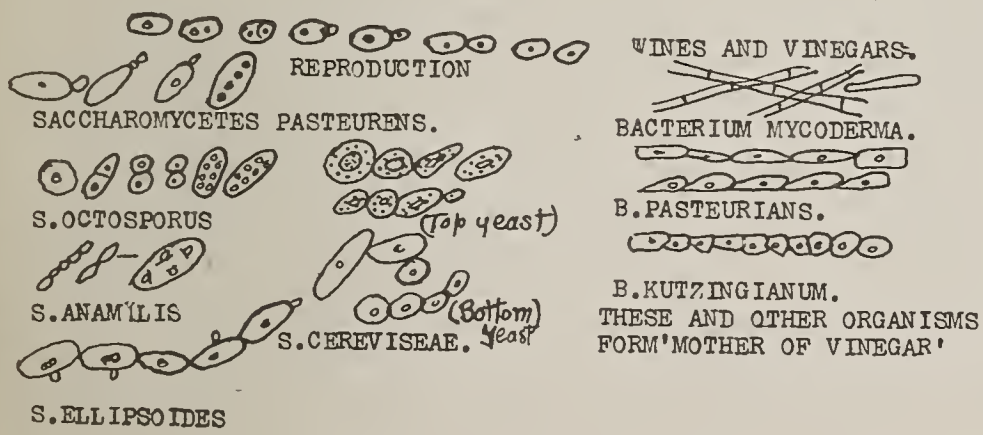
The following are very pathogenic to man:

Odium—a bridge between yeasts and molds.

Odium lactis causes the souring of milk and butter. The branches of hypha break up into shorter rod like spores, and appear in milk as white mold.

Odium albicans is found in the membranes of the mouth. It grows on the surface of media; but the hyphae and mycella may pénetrate deep into the host tissues. It produces white mouth, or thrush in man.

Odium mycosis, or plastomycosis, produces wart like lesions, which contain small abscesses. It may infect large areas of the skin, lungs and kidneys and resembles in habit the yeasts.



EXERCISE

1. Place a drop of water on a slide, add a drop of iodine solution, mix, then add a small amount of the yeast, mix well, cover and examine, the starch will be stained blue black, the yeast cells, much smaller, are brownish. Draw.

2. Stain another specimen of yeast with C. Z. I. Make a permanent mount.

TEETH AND BACTERIOLOGY OF THE MOUTH

Teeth are necessary for mastication, articulation and ornamentation.

Man has two sets of teeth; the first or temporary, deciduous or milk teeth, numbering 30. The second set, or the permanent teeth, number 32, four of which are erupted while the first teeth are in position, these are the six year molars.

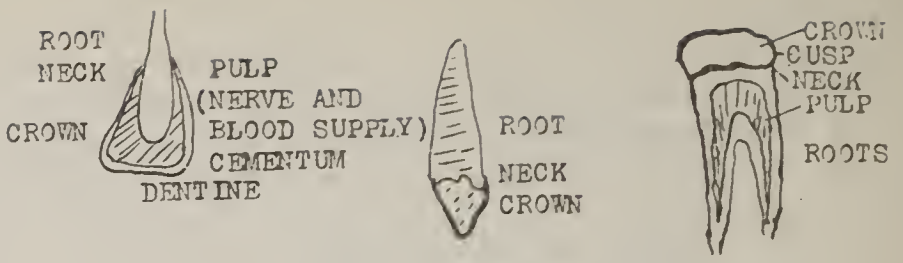
Caries or decay is one of man's worst enemies, and is caused by first, neglect or carelessness in properly cleansing the teeth; and second, accidents, which are rare.

When micro-organisms enter the dentine breaking down the tissues, a hyperemia (tooth ache) ensues which may be arterial or nerve (jumping).

The mouth being the channel for food, is also the main channel for the entrance of micro-organisms. Unless the teeth and mouth are properly cleansed after each meal and before retiring, the food which lies between and around the tooth will ferment, and a production of lactic and other acids results, which attack and break down the enamel—the tooth covering, thus forming a ready entrance for the omnipresent micro-organisms. The fermenting food and its products, along with the heat and moisture furnished by the oral cavity, and especially at night, during sleep, furnish the quietness required by micro-organisms to thrive; these all form a perfect culture medium and incubator for the micro-organisms to multiply and rapidly grow.

Teeth should be cleansed thoroughly before retiring and after each meal. It should take at least five minutes to clean the teeth properly.

Brush up and down, that is brush over the gum and down the teeth in the upper jaw and over the gum and up over the teeth in the lower jaw. Simply brushing across the teeth will not cleanse between the teeth, and that is where decay first begins. This will also prevent tartar from forming, and receding gums. Tartar forms rapidly in acid mouths; and microscopical examinations of tartar show it to be composed of deposits of calcium carbonate, many epithelial cells, leukocytes and micro-organisms. Among the micro-organisms which were found in the tartar are motile spirochetes, segmented bacilli which form in long bands, and stain red-blue with iodine, leptothrix bucallis, short forms of bacilli, micrococci and many others. Almost any known micro-organism may be found in the mouth, or in the tartar. The mouth usually is bacterially the dirtiest place in the body.



THE STRUCTURE OF TEETH.

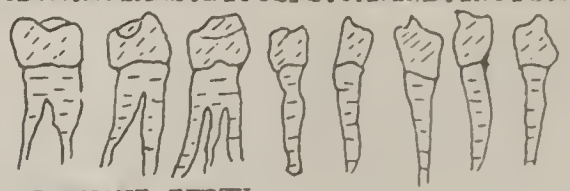
UPPER MAXILLARY

MILK MOLARS CANINE INCISORS.

LOWER MANDIBULAR

TEMPORARY TEETH.

WISDOM.MOLARS.BICUSPS.CANINE.INCISORS.



PERMANANT TEETH.

PYORRHOEA(RIGG'S DISEASE)
TOOTH BECOMES DISEASED,GUMS RECEED,TOOTH LOOSENS.

Your teeth are more valuable to you than pearls; hence treat them accordingly. Have your teeth examined every six months by a competent dentist. This will avoid large dental bills and many painful hours in the dental chair.

Tooth powders, pastes or liquids should be of an alkaline nature, free from syrups, or other easily fermentable substances, alum, charcoal, minerals and mineral acids of all kinds, (except boric), salicylates, salol, potassium compounds, etc.

They should be non-caustic, non-decalcifying, non-poisonous and of a sufficient germicidal power. Rarely one preparation answers all of these requirements; usually if the preparation is of a good color, odor and taste, the public is satisfied, and if of sufficient profit, the pharmacist is satisfied.

A brush with bristles as stiff as the individual can use, without causing irritation to the gums, and with a tuft at the end for cleansing around the back teeth, and the bristles set firmly in the handle, is the best brush to use. Loose bristles may be swallowed and cause complications, such as lodging in the appendix, there causing irritation, pus production, and operation and a large surgeon bill.

One of the best tooth preparations for cleansing and whitening the teeth and making the mouth alkaline is sodium bicarbonate. If this is used after meals and before retiring it will preserve good teeth, but will not restore decayed teeth. A good wash consists of Sulphocarbolate of Zinc 4 grams. Alcohol 30 mils, Distilled Water 60 mils and True Oil of Wintergreen ½ mil. Or one of the best mouth wash antiseptics with a very high germicidal powder, is a saturated aqueous solution of thymol, which will prevent the growth of the majority of the mouth bacteria and also has a pleasing taste.

It has been stated that only 2% of the population of the United States use a tooth brush; and this is borne out in the statistics. For example: 30,000 mouths were examined—there were 18,000 extractions necessary; and 60,000 cavities caused by decay, which were past filling, and could have been prevented with proper care. If each one caused an average loss of time and cost of repair at $2.00, it would mean a waste of $156,000, which could have been saved by timely care. In New York City schools in 1917, there were 76,000 children who failed to be promoted on account of bad teeth, and these children cost the City of New York $1,037,607.00 to duplicate the year's schooling.

Tooth preparations containing alkaloids of ipecac may be of some value in Rigg's disease, but it is doubtful if they contain sufficient alkaloids to be of real value. The alkaloids are taken in tablet form for entamebic dysentery and are injected into the pus pocket in diseased teeth.

THE HIGHER BACTERIA

Show a distinct advance over the lower group. They are closely related to molds and have filaments which usually are in segments and sometimes a capsule surrounds the whole bacterium. The term given to the higher bacteria is Chlamydobacteriaceae and it is subdivided into:

1. Cells without sulphur granules.
   A. Filaments unbranched.
      a. Cell division in one direction. Leptothrix.
      b. Cell division before gonidia formation in three directions.
         1. Filaments with faint sheath. Phragmidiothrix.
         2. Filaments with distinct sheath. Crenothrix.
   B. Filaments which show false branching. Cladothrix.
2. Cells containing sulphur granules. Thiothrix.
3. Long, motile filaments. Beggiatoaceae.
4. Spirals or rods, break up into cocci. Spirulina.
5. Felt like mycelium, branching. Streptothrix.

BACTERIA OF THE MOUTH

Many of the bacteria which are found only in the mouth will grow only in saliva, in which the teeth are constantly bathed. There is about the same amount of saliva in 24 hours as urine. The froth in the saliva is caused by the parotid gland, which is located below the ear.

THE MOST IMPORTANT BACTERIA INDIGENOUS TO THE MOUTH

Cladothrix occur as spherical, rod or filamentous forms and show pseudo or false branching. They reproduce by arthrospores.

Leptothrix. This genus belongs to the higher Schizomycetes (which are able to produce thread like forms). They are rod shaped, spherical or filamentous in form. The filamentous forms show a differentiation between the base and apex. The filaments may be either straight or spiral. Reproduction by spores does not take place.

Spirulina—Rod shaped cells, sometimes in spirals; the threads break up into cocci like bodies and it reproduces by arthrospores (the whole cell becomes a spore).

Streptothrix appears as felt like mycelium filaments and shows true dichotomous branching. Club shaped thickenings appear at the end of some of the threads and various forms

are produced by the breaking up of the threads, simulating cocci, bacilli and spirilla. These form new individual streptothrix.

ALMOST EVERY KNOWN MICRO-ORGANISM MAY BE FOUND IN THE MOUTH.

Leptothrix grow in the form of long threads and are subdivided into:

Leptothrix innominata is found in the soft white deposits on the teeth. When the white deposit is examined under the microscope, large rounded masses are seen, with thin margins and somewhat zigzag thread like organisms protruding. These thread like formations are twisted, non-motile and inarticulate. The white masses when stained with tincture of iodine, acidulated with lactic acid, are seen to consist of epithelial cells, masses of micrococci and many rod and thread like organisms, which stain a yellowish color; while many of the other bacilli are colored blue-violet.

Bacillus or Leptothrix buccalis maximus appears as isolated thread like bacillus, single or in tuft like masses; some individuals may be 150 microns in length, while the rod like forms are rarely over 10 microns in length. It is the largest bacterium occurring in the mouth. It stains a brown-violet with tincture of iodine.

Leptothrix buccalis maximus is found in deposits on the teeth and are long straight or curved filaments with short joints. They do not stain with tincture of iodine.

Leptothrix giganta is associated with pyorrhoea alveo- the teeth or a creamy layer along the gum margin. When mounted in glycerin, or in a hanging drop with saliva or in salt solution, they appear as felted masses composed of entwined thread like forms; the projecting threads are finger shaped, with cocci bodies attached to the thread by basidia. When stained by the Gram method, the beaded appearance of the thread like organism is seen.

Leptothrix gigantea is associated with pyorrhoea alveolaris and appears as tufts with many diverging threads. It forms cocci, rods and threads. The threads vary in thickness and may be straight or curved.

Bacillus luteus is found in dental caries. It is a non-motile, Gram positive organism. It grows on gelatin media as minute punctiform non-liquefying colonies in two days; later the colonies become larger, rounded and golden-yellow. It is slightly pathogenic for animals, producing a local reaction only.

Bacillus bucalis minutus, is a very short bacillus, with rounded ends, almost as broad as long. It is an aerobic, liquefying, chromogenic organism. It grows on gelatin media as slightly elevated, yellow, liquefying colonies.

Bacillus buccalis fortuitus has square ends, often united in pairs, and at different angles. It is an aerobic, liquefying, non-motile organism. It grows on gelatin media as small, round, liquefying colonies.

Jodococcus vaginatus occurs in large numbers in unclean mouths and appears singly or in chains of from 4 to 10 cells in length. The cells are enclosed in capsules and resemble disks or squares. The cells do not stain with tincture of iodine, but their contents are colored a dark blue-violet. Jodococcus magnus grows on starch-gelatin-salvia-agar.

Spirillum sputigenum is found in all mouths but in greater numbers in unclean mouths, and especially in mouths with inflamed gums. It occurs in the shape of comma curved rods, which show a very active spiral movement, sometimes short spirals or S. forms may be seen. It is an aerobic facultative anaerobic, liquefying, motile spirillum; growing on gelatin media at 22 degrees C. as minute gray-white colonies, which are moist and flat, and soon liquefying the surrounding gelatin. On agar media the colonies are brownish, smooth, moist and flat. They are pathogenic for guinea-pigs, causing death in three days.

Spirochaeta dentium is found under the gum margins in cases of gingivitis marginalis. It occurs in long spirals, from 8 to 25 microns in length; the spirals are unequal in distance and thickness; and show motility.

Streptothrix buccalis is found in the white deposits on teeth, in pyorrhoea pus and in gingival inflammation. It occurs in filamentous forms, which show lateral branches, with frequent club shaped ends. They grow on gelatin media at 22 degrees C. as minute, hard, raised, colorless, somewhat cone shaped colonies, which soon liquefy the gelatin. On agar media the colonies develop the cone shape and often crack across the summit. The cultures give off a characteristic odor resembling that of a damp musty cellar.

MOUTH BACTERIA WHICH GIVE A BLUE OR VIOLET STAIN WITH IODINE

Bacillus buccalis maximus.

Jodccoccus magnus which occur as large cocci or diplococci in masses in the soft deposits upon the necks of the teeth.

An unnamed small micrococcus which stains a blue-violet color.

An unnamed micrococcus, which stains a pink color.

Some of the yeasts.

THE NON-CULTIVABLE PATHOGENIC MOUTH-BACTERIA are: Leptothrix innominata, Bacillus buccalis maximus, Jodococcus vaginatus, Spirillus sputigenum and Spirocheta dentium. Although these bacteria are found in all mouths and may be found in pure cultures, it is impossible to cultivate artificially

THE CULTIVABLE PATHOGENIC MOUTH-BACTERIA are:

The micrococcus of sputum septicaemia, appear as capsulated oval cocci; single, pairs or in short chains. It grows on blood sugar media as a nearly transparent gray-white gelatinous coating. In broth the growth sinks to the bottom and resembles a sandy precipitate, while the liquid remains clear. The organisms when injected into mice and rabbits will cause death within 36 hours.

Bacillus crassus sputigenus, is a short thick bacillus, straight, curved or twisted, growing on gelatin media as grayish-white spots, which are elevated above the surface and later appear as large greenish-white rounded slimy drops, rising high above the surface. In stab cultures the colonies appear as a nail shaped growth. These organisms kill mice in 24 hours but do not kill rabbits unless the injections are intravenous, when the rabbit dies in 48 hours, from a form of septicemia.

Staphylococcus pyogenes aureus and albus and streptococcus pyogenes are the chief pyogenic organisms found in the mouth.

Micrococcus tetragenous occur in the form of capsulated cocci which by fission form groups of fours. It grows on gelatin media as white dot like colonies, which are granular and glassy. It will kill mice and guinea pigs in less than 10 days, even when the saliva containing these organisms is injected, death occurs in this time.

Bacillus salivarius septicus is found in practically all mouths and it is in the form of very short elliptical rods with the extremities tapering. It is very difficult to cultivate. When rabbits or mice are injected with 0.5 mil of saliva containing these organisms, death results in less than 72 hours.

Streptococcus septo-pyoemicus is probably a sub-specie

of the organism which is found in erysipelas. It is very pathogenic for rabbits and mice, causing a chronic septicemia, with a pus formation at the site of injection.

Micrococcus gingivae pyogenes appear as irregular cocci or very short thick rods, single or in pairs. It grows rapidly on nutrient gelatin as rounded, distinct, sharp margin colonies, which at first are of a gray-white color and becoming darker as growth proceeds. It will not liquify gelatin nor produce acid in sugar broths. When injected into animals, an abscess is produced, followed by necrosis. When injected intraperitoneally death is produced in less than 12 days.

Bacterium gingivae pyogenes appear as a short thick rod with rounded ends. It grows on ordinary media, as rounded yellowish-green colonies, and it will liquify gelatin within 48 hours. Producing death in mice when injected intraperitoneally within 24 hours. Subcutaneous injections result in abscess formation.

Bacillus dentalis viridans is found in superficial layers of carious dentine. The bacillus is a slightly curved, pointed rod, single or in pairs. It grows on ordinary media as rounded, sharp-edged slightly yellowish colonies, which show several concentric rings. The culture media becomes a greenish color due to the color produced by the bacillus. Intraperitoneal injections in animals produce death within 6 days.

Vibrio Finkler-prior (sometimes called spirillum) because of certain diarrhoea atacks. They are small short curved bent rods, as the comma, or in threads, and stain Gram negative.

THE AMEBA

Entameba histolytica is an ambeoid organism which is found in large numbers in intestinal discharges of persons suffering from a form of tropical chronic dysentery. It measures from 15 to 50 microns in diameter, with a single, rounded, vesicular nucleus and vacuolated protoplasm. See pages on Entameba.

Entameba coli is found in normal intestins and multiplies by both fission and spore formation.

Entameba gingivalis resembles E. histolytica, but is much smaller, less active and less motile.

Entameba buccalis is found in cavities of carious teeth.

THE ACID FORMING BACTERIA FOUND IN DENTAL CARIES

1. Deep layers of carious dentine.
   Streptococcus brevis, Staphylococcus albus and B. necrodentalis.
2. Superficial layers of carious dentine.
   Streptococcus brevis, Staphylococcus albus and aureus, Sarcina lutea, albus and aurantiaca.

THE DECALCIFYING BACTERIA

1. Deep layers of carious dentine.
   None isolated.
2. Superficial layers of carious dentine.
   B. mesentericus-rubra, vulgatus and fuscus, B. furvus, B. gingivae pyogenes, B. liquefasciens flourescens motilis, B. subtilis, B. plexiformis and various proteus.

BACTERIA WHICH ARE PRACTICALLY ALWAYS PRESENT IN DENTAL CARIES

B. gangraenae pulpae, Staphylococcus pyogenes, Staphylococcus albus, aureus and Sarcinate lutea

SAPROPHYTIC BACTERIA FOUND IN THE MOUTH

Bacillus coli commune, Bacillus luteus, Bacillus buccalis minutis, Bacillus buccalis fortuitus, Bacillus of Vignal, Vibro Finkler-Prior and Micrococcus roseus.

THE STRUCTURE OF A BACTERIUM

A thin wall enclosing a clear and as far as known, structureless content. The cell wall is allied to cellulose and is of a radiate honey comb appearance. The coloring matter of the chromogenic bacteria is in the wall. Sulphur granules of the Beggiota are in the inner plasma. The plasma contains highly refractive granules which are not spores. Most bacteria are surrounded by a capsule—a homogenous gelatinous or albuminous like mass. Various kinds of bacteria produce within their plasma, highly refractive bodies, which are capable of withstanding high temperatures, dessication and strong disinfectants; these are the spores. Usually one spore is formed to the bacterium. Spores may remain in a dried condition for years and when brought under the proper conditions grow and multiply.

It is estimated that one bacterium, two microns long and one micron wide (usually large) weighs 0.000,000,001,571

ALMOST ANY KIND OF MICRO-ORGANISMS MAY BE FOUND.

MICRO-ORGANISMS FREQUENTLY FOUND IN THE MOUTH,

mgm. and will reproduce itself under proper conditions by binary fission once every 30 minutes; would in two days number over 281 billion and occupy a volume of ½ liter.

Bacterial development is hindered by lack of proper food, moist temperatures, activities, products of other bacteria, sunlight, light, motion, et al.

EXERCISE

1. Make five slides as previously instructed, using the material from the gum margin.

2. While these slides are drying in the air, make a hanging drop, after examining carefully remove the cover glass and stain as directed for the tubercle bacillus (see pages on Consumption).

3. Stain one of the air dried slides with Loeffler's alkaline methylene blue, for 1 minute, wash with water, dry and examine; if the stain and specimen are good, mount in canada balsam, by placing 1 drop on the center of the cover glass and pressing the inverted cover glass over the specimen of the slide. The slide and cover glass must be dry, or the balsam will become cloudy. Use the oil immersion lens.

4. Stain another slide with aniline gentian violet, stain for one minute, be exact in your time, wash in water, counter stain for one minute with Loeffler's stain. Examine, and if good, dry and mount. Make drawings.

5. Stain one slide by the Gram method. If good, dry and mount. Draw.

6. Scrape the inside of the cheek and make a slide of the scrapings; air dry, flame, stain with an aqueous solution of eosin for 30 seconds; wash in water, and counter stain with Loeffler's stain for one minute, wash, dry, examine and mount.

Make a full and complete report and drawings. Classify the bacteria as to shape; do this for each slide. Keep your microscope clean and dry. After drawing under the high power, use the oil immersion lens. The lens is immersed in a drop of rectified cedar oil, placed over the specimen and on the cover glass. The higher the specimen is magnified, the more light is needed; hence this oil is used to keep the rays of light from being dispersed. Take good care of this lens for it is very expensive and easily damaged.

MICROSCOPICAL EXAMINATION OF SPUTUM

If saliva is permitted to stand for several hours, it will separate into two parts; the upper part is clear, but the lower part is cloudy and contains salivary corpuscles which resemble leukocytes but coarser and larger,—payment epithelial cells which are large, irregular outlined, somewhat polygonal, with a well defined nucleus and nucleoli and always many micro-organisms.

Curschman's spirals are found in the sputum especially in asthma. Leukocytes, elastic tissue, protozoa, yeasts and molds.

COATING ON THE TONGUE

To examine the tongue coatings, a diphtheria swab or platinum needle may be used to collect the material for placing on the slides.

Brown coating contains epithelial cells, many micro-organisms and desquamated epithelial cells.

White coating shows in addition to the above a few salivary corpuscles.

The most frequently found micro-organisms are bacillus diptheriae, staphylococcus aureus, streptococcus, pneumococcus, micrococcus catarrhalis and in mucous patches in syphilis, the spirochoeta pallidum.

NASAL SECRETIONS

Normal nasal secretions should be transparent, colorless, odorless, tenaceous, and with a saline taste and alkaline reaction.—And contain pavement epithelial cells, ciliated epithelial cells, some leukocytes, many micro-organisms, pus cells and almost any known micro-organism.

TESTING FOR ENTAMEBA IN RIGG'S DISEASE

The entameba which are associated wtih this disease have vacuoles into which food is taken by osmosis from the leukocytes, and also engulf the leukocytes. The vacuoles store the food and the entameba by its own secretions as enzymes, assimilates the food as needed; and this taking of food is supposed to cause the shrinking of the gums.

With a clean toothpick scrape the material from a scaler on to a clean slide, spreading it out in a thin layer with the flat side of the toothpick—care being taken to hold the toothpick lightly and not to rub the material back and forth or the entameba will be crushed.

The scaler must be used to scale the tooth in the diseased pocket, or in the receding gum cavities. Dry the slides in the air.

1. Flame the slide until the slide feels hot to the back of the hand.

2. Apply Czaplewski's carbol fuchsin stain for 5 to 10 seconds; wash the slide in water.

3. Counter stain with Loeffler's stain for 5 seconds; wash the slide with water and examine the slide against a light background; and if not sufficiently stained repeat the staining for 5 seconds and so on until properly stained.

4. Wash well with water, blot, air dry and examine with the oil immersion lens.

Material from the proper lesions will show many red blood cells, pus cells, with many large cells from the granulating surface, many bacteria, many spirochetes and a large number of entameba. The red blood cells stain a deep red, the pus cells a bright purple with irregular shaped nucleus with pink protoplasm. Bacteria and spirochetes stain as the species, a blue, purple or bright red.

The entameba usually vary in size from that of a pus cell to four times as large. Their endoplasm stains a deep blue and is surrounded by the slightly irregular border of purple stained endoplasm. A small round or oval nucleus, stains a deep port wine color and is centrally located, along with one to twelve inclusion bodies of neuclear material which stain a deep purple or black. These inclusive bodies are contained in vacuoles, which are shown by a clear ring inclosing each one. Usually the whole entameba appears surrounded by a clear zone showing the retraction of the whole, caused by drying.

In 70 cases of Pyorrhea alveolaris, the following organisms were found: In 19 cases streptococcus, in 5 cases pneumococcus, in 14 cases bacillus necrosis-dentalis, 3 cases micrococcus catarrhalis, in 15 cases bacillus septus, in 12 cases staphylococcus aureus, in 5 cases saccharomyces, and in 6 cases microccus citreus-granulosis.

MICROSCOPICAL EXAMINATION OF VOMITED MATERIAL

Permit the material to stand until small tapioca like bodies collect at the bottom of the vessel. When these are examined they are found to contain many shell like formations, single or groups; this is mucin. There are epithelial cells from the lining of the ducts, the goblet cells. Some micro-organisms, including those which inhabit the mouth, yeasts, Boas-Oppler bacillus from patients suffering from carcinoma of the stomach. This organism resembles very

closely and may be identical with the bacillus bulgaricus. Tubercle bacillus, sarcine—in squares, protozoa, blood cells, pus, and food particles as muscle fibres, starch, fat globules, and various food particles.

THE BLOOD CELLS

"The blood is the life" and consists of two chief parts, the plasma and corpuscles. The corpuscles are two kinds—the white and red blood corpuscles.

The blood of man contains about 5,000,000 red blood cells—Erythrocytes, from erythros—red and cytos—cell, and about 5,000 white blood cells—Leukocytes from leuko—white and cytos—cell. to the cmn. of blood.

	Hemoglobin	Erythrocytes	Leukocytes
Man	90 to 100%	5 to 5½ million	5 to 7,000
Woman	80 to 100%	4 to 5 million	5 to 7,000
Child	70 to 80%	4 to 5 million	3 to 9,000

THE ERYTHROCYTES

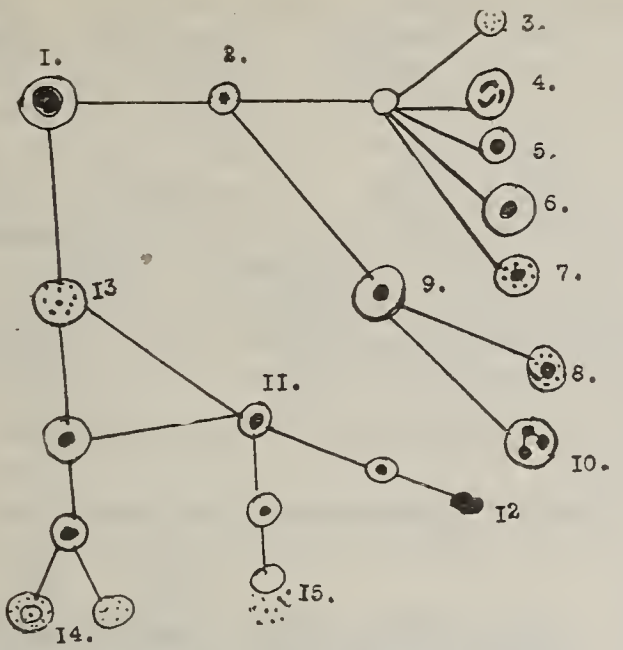
Erythrocytes are non-nucleated, rounded, biconcave disks about 1/3200 by 1/1200 of an inch. The number of erythrocytes bears no relation to the quality of the blood; as 2,000 feet altitude increases the number one million; profuse sweats and diarrhoea, splenic enlargement and other diseases increase the number. The erythrocytes constitute about 14% of the whole blood and about 86% of the solid content.

Their function is the carrying of oxygen, and they stain with the acidophilic stains such as the red or yellow stains. They are disintegrated and liberate pigments consisting of hemoglobin and others, which are converted to bile pigments by the liver. It is thought the worn-out cells are destroyed by the spleen.

Macrocytes are larger than the normal erythrocytes; the normal erythrocytes measure about 0.5 micron, while these cells are from 16 to 20 microns and are found in grave forms of anemia. They are polychromatic (many colored) in staining.

Microcytes are smaller than normal erythrocytes and are found in less grave forms of anemia.

Poikilocytes—A term given to distorted erythrocytes, often from faulty technic.



1.PRIMARY WONDERING CELL, 2.MYELOBLAST,3.EOSINOPHILLA (EOSIN THE RED DYES AND PHILLIA,LOVING),4.POLYMORPHO NUCLEAR LEUKOCYTE(MANY FORMED NUCLEUS,WHITE CELL),5. LARGE MONO NUCLEAR LEUKOCYTE(MONO ONE),6. LARGE MONO NUCLEAR LEUKOCYTE, 7.MYELOCYTE, 8.EOSINOPHILIC LEUKOCYTE, 9. LARGE MONO NUCLEAR LEUKOCYTE,I0. GRANULAR TRANSITIONAL LEUKOCYTE,II.ERYTHROBLAST, I2.ERYTHROCYTE(RED CELL),I3. ADULT MARROW CELL,I4. NORMOBLAST, I5. HAEMODOBLAST OR BLOOD PLATLET OR SAND.

THEORY OF BLOOD CELL FORMATION.

LARGE MONONUCLEAR LEUKOCYTE OF NORMAL BLOOD ↑

LARGE LYMPHOCYTES ← LYMPHOCYTES → PATHOLOGICAL BROAD BODIED MYELOCYTES,MAY HAVE INDENTED NUCLEUS.

SMALL YOUNG LYMPHOCYTES ↓ MICRO LYMPHOCYTES ↓ LEUKOBLAST

OLDER LYMPHOCYTES OF NORMAL BLOOD WITH INDENTED NUCLEUS OR BROAD BODY. ↓ MICRO LEUKOBLAST → PROMYELOCYTE ↓ MYELOCYTE

OLDER BONE MARROW LYMPHOCYTES WITH BROAD BODY. ↓ MICRO PROMYELOCYTE. ↓ METMYELOCYTE ↓ POLYMORPHO NUCLEAR LEUCOCYTE.

PAPPENHEIM'S THEORY.

Crenation and vaculation are due to poorly prepared slides.

Pathological erythrocytes.

Polychromatophilla (many color loving). These cells stain a dirty blue tint. Granular, basophyllic (basic loving —take the blue or brown stains) degeneration which shows as blue dots on a pink ground—Found in severe anemia, malaria, lead poisoning, chancre, etc.

Erythroblasts, are normal nucleated cells if they come from the bone marrow; but they are pathological if they are found in the peripheral circulation. They are about as large as the normal cell, but contain an excentric nucleus which stains intensely with basic dyes, often appearing blackish and like the setting in a ring.

Microblasts are erythroblasts which are smaller than the normal erythrocytes.

Megaloblasts are erythroblasts which are two to three times as large as the normal erythrocyte.—Found in pernicious anemia.

Normoblasts. The nucleus stains less intensely, is very large with a radiating structure, distinct but irregular in shape.—Found in secondary anemia or leukemia.

Gigantoblast.—Are very large erythroblasts and are found in aplastic anemia, which is a very severe form of pernicious anemia; here the nucleated cells are rarely found.

Blood platelets are normally present in about 350,000 to the cmm. They disintegrate rapidly after the blood is drawn. They probably are pinched off portions of the bone marrow giant cells. They do not contain hemoglobin.

Eosinophillic, eosin loving.—When the cells are alive they do not take the stain, staining only when they are dead.

Basophyllic, basic loving.—Take the blue or brown stains.

Chlorosis-green sickness.

Anemia.—The blood is deficient in red coloring matter, or hemoglobin, which is carried by the erythrocytes; thus an insufficient supply of oxygen is furnished to the body.

Hemoglobinemia.—A secondary anemia, where the erythrocytes are reduced in number.

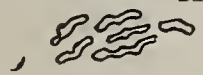
Pernicious anemia.—The pathological form.

NORMAL ERYTHROCYTE. MICROBLAST. NORMOBLAST. MYELOBLAST.

ERYTHROBLAST.

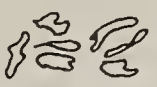
BLOOD PLATELETS OR SAND.

DEFORMED CELLS

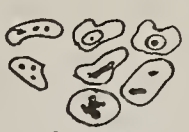


MICROCYTE

MYELOCYTE



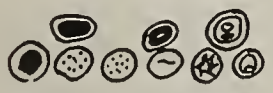
POIKILOCYTE



DEGENERATED.

CRENATED. (OFTEN DUE TO FAULTY TECHNIC)

ERYTHROCYTES. (ERYTHROS-RED, CYTOS-CELL)

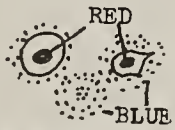


SMALL LYMPHOCYTES

LARGE LYMPHOCYTES

EOSINOPHILES
(TAPE WORMS ETC)

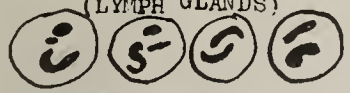
MAST CELLS



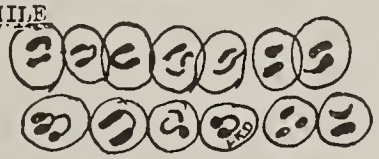
MYELOCYTE.

LARGE MONONUCLEARS
(LYMPH GLANDS)

BASOPHILE



POLYNUCLEARS.



POLY MORPHO NUCLEARS.

POLY-MANY, MORPHO-FORMED, LEUKO-WHITE, CYTOS-CELL.
NEUTROPHILES-NEUTRAL IN STAINING, REDDISH NUCLEUS AND IRREGULAR OR OVAL IN OUTLINE. TRANSITIONALS ARE FAINT IN OUTLINE, FAINT HORSE SHOE SHAPED NUCLEUS OR MULTILOBAR. MAY BE NEUTROPHILIC IN STAINING. BASOPHILLIC-BASIC STAINING, THE BLUES OR BROWNS. ACIDOPHILLIC-ACID STAINING, OR EOSINOPHILIC, STAINING RED OR YELLOW.

THE LEUKOCYTES.

THE LEUKOCYTES

The word means leuko, white, and cytos, cell. The leukocytes are larger than the erythrocytes and contain nuclei. They are round and average about 1/25000 of an inch in diameter. They are basic in staining, staining blue or brown. They are divided as follows:

1. Small lymphocytes. These are small cells about the size of the erythrocyte, with a very large centrally located, deeply staining nucleus, many times almost filling the cell, and surorunded by a narrow zone of cytoplasm. The nucleus may be crescent or other formed and vacuolated. They are found in excessive numbers in lymphatic leukemia and are associated with a great increase in numbers of the leukocytes The nucleus usually stains a deep violet color.

2. Large lymphocytes.—Possess more cytoplasm. The nucleus is more rounded and is not stained so deeply as that of the small lymphocytes. The cytoplasm stains pure blue and may contain azure colored granules. These cells are common in children's blood.

3. Large mononuclears (one nucleus)—Are large, round or oval cells; the nucleus is not so deeply stained as that of the lymphocytes. The nucleus is frequently irregular in outline and there is no sharp distinction between the nucleus and the cytoplasm. The cytoplasm contains no opaqueness, but is of a frosted appearance, similar to frosted glass (difference from lymphocytes).

4. Transitionals are a late stage of decay of the mononuclears,—the nucleus is more idented; frequently horse shoe in shape, or irregular in outline and of a washed out violet shade, much less intense than that of the large mononuclears.

5. Polymorphonuclear leukocytes. (Poly—many, morpho—formed.) These cells have many different shaped nuclei and constitute about 70% of the leukocytes. In infections when a high percentage of these cells is found in the blood the prognosis is good; but if there is a decreased number the prognosis is very bad.

These cells are often referred to as the Lymphatic series. The large mononuclears and the transitionals are the cells which are phagocitized as in malaria. The lymphocytes take their origin from the lymphoid tissue, and probably the young cells are larger and more immature than the small lymphocytes. There are also narrow, gland and granular lymphocytes.

Neutrophyllic leukocytes. These cells have fine lilac stained granules,—neutral in staining. The single nucleus is shaped like the kernel of a walnut or may resemble an S or Z. They are derived from the bone marrow.

Polymorphonuclear neutrophiles. These cells are about two times as large as the erythrocytes. The protoplasm is neutrophilic in staining, the granules are usually stained red and the nucleus blue.

Iodiophile granules, or the glycogen, are found in certain suppurative diseases.

Eosinophile leukocytes, Eosinophila.—Cells with coarse refractive granules which stain a bright pink—the acidophila or acidophile granules. The cells are a little larger than the polymorphonuclears. The normal eosinophiles are distinguished from the eosinophilic myelocyte by their having two distinct lobes to the nucleus, while the nucleus of the myelocyte is rounded. These cells are frequently increased in numbers by intestinal parasites. An increase of 1 to 2% in childhood is considered as normal. Also the natives of China have 15 to 20% eosinophiles and are considered as normal. Skin diseases, cancers, tumors, asthma and intestinal parasites increase the number.

Mononuclear eosinophiles are pathological, and are best considered the same as eosinophilic myelocytes.

Polynuclear eosinophiles are considered as normal. These contain two or three nuclei.

Basophilic leukocytes or Basophiles or Mast cells. In these cells the nucleus occupies about two-thirds of the cell. The nucleus stains blue or brown. The coarse granules stain a deep violet-blue. In fresh blood the granules are not so well defined. The trilobed nucleus stains less intensely than the granules. These cells are about the size of the polynuclears, and are a pathological indication if found in increased numbers in the blood stream.

1. Neutrophilic myelocytes.—Are large cells, with a centrally placed feebly stained nucleus, which on account of it being so large, is hard to distinguish from the cytoplasm, which seems to merge with it. The cytoplasm is distinctly dotted with neutrophilic granules. There is no distinction between the regular or irregular indented mass or nucleus—different from the large mononuclears. The cytoplasm is slightly neutrophilic in staining. At times basophilic and neutrophilic granules may be found in cells from the bone marrow.

2. Eosinophilic myelocytes.—Eosinophilic marrow cells or mononuclear eosinophiles are distinguished from normal eosinophiles by having a single rounded nucles (not bi-lobed.—Found in spleno-myelocytosis leukemia). One or more are generally found in any leukocytosis. If they are found in diphtheria, the prognosis is bad.

3. Non-granular marrow cells are large delicate cells, containing homogeneous protoplasm which as well as the nucleus stains faintly basophilic.

4. The irrigation cell of Turch or the Plasma cell, has a faintly stained excentric nucleus and a dark opaque, frequently vacuolated cytoplasm, and is usually recorded in blood counting as large mononuclears. If found after a leukocytosis they are pathological.

In pernicious anemia the giant cells are less abundant as are also the blood platelets. But in myelogenous leukemia, many are found. Theny range from 2 to 5 microns and are stained a purple color, showing like projections. They are often confused with Keokemia, which appear as highly refractive bodies, showing oscillatory movement and are supposed to be cast off fragments of the red blood cells, and often appear as protozoal bodies and have been taken for the malarial protozoa.

Leukopenia is a term used to designate a reduction in the normal number of leukocytes, as 4,500 represents a slight reduction in some cases but 2,000 indicates a marked leukopema, as seen in acute miliary consumption.

A moderate leukopenia exists in chronic alcoholics and arsenical poisoning. There are many causes for a reduction such as pernicious anemia and Rant's disease.

Phagocytes. (Eeating cell). The leukocytes which throw out protoplasm and engulf foreign substance.

Macrophage is polynuclear and comes from the bone marrow. It reabsorbs the foreign erythrocytes which enter the body as in meats; they also throw out protoplasm and engulf bacteria. Somethimes they eat to bursting as may be seen in malarial blood where the rapid reproduction of the parasites causes the macrophage to burst,—it is at this stage the malarial manifestations are seen.

Eosinophilia, an increase in the number of eosinophiles.

Leukocytosis, an increase in the number of polymorphonuclears, lymphocytes, etc. This term is employed where the white blood cells are increased in number.

Lymphocytosis.—When an excess of lymphocytes is present.

If in several infections, good resistance is shown by an early and persistent leukocytosis, the prognosis is good. If there is a slight infection and slight resistance there is no leukocytosis. In fulminating infections (boils, etc.) there is usually no leukocytosis, but a high percentage of polymorphonuclears. A light infection and good resistance may not produce a leukocytosis. Small pox at the time of pustulation, scarlet fever, liver abscesses, plague, erysipelas, meningitis, etc., produce about 12 to 15,000 leukocytes, and in spirochetal fevers as relapsing, a leukocytosis of 25 to 50,000 is found.

Lymphocytosis is most marked in lymphatic leukemais and hereditary syphilis. Whooping cough produces a lymphocytosis of 20 to 30,000. Children normally have an excessive production of lymphocytes. Enlarged tonsils (these are the filters for all the air passes over the tonsils, and many of the bacteria are filtered out, before the air passes to the lungs, they also furnish antidiphtheritic serum)—should never be removed; if they are diseased the diseased portion only should be cut out. They show 10 to 15,000 leukocytes of which 50% will be lymphocytes. This is true also of rickets, scurvy, etc.

Anemia-primary. A lack of hemoglobin in the erythrocytes. Many of the erythrocytes are oval or hour glass shaped.

A normal leukocytosis is found as follows: In uncomplicated tuberculosis, influenza, measles, malta fever, syphilis, sleeping sickness, chlorosis, etc. Malaria at the time of the rigor shows a large increase of leukocytes and transitionals.

Anemia—simple primary. Between chlorosis and pernicious anemia. Abnormally large erythrocytes are found.

Anemia—secondary. This form may be definitely traced to some disease, as syphilis, carcinoma, consumption, etc. Normoblasts are found in small numbers, seldom megaloblasts are found, except in parasite anemias as the Russian tape worm. The erythrocytes number from 2 to 4,000,000, thus differing from chlorosis. Leukocytes are frequently increased to 15,000. Splenic anemia shows marked leukopenia; malignant diseases show moderate leukocytosis. Sarcoma shows a large number of eosinophiles. In secondary anemia the erythrocytes are usually distorted in outline.

Anemia—pernicious. On puncturing the blood appears normally colored. The yellow marrow of the long bones is transformed into a soft bright yellowish or red lymphoid tissue, containing a great number of megoblasts. Areas of fatty

degeneration are characteristic, and tiger lily spots of the heart muscles are especially diagnostic. Iron (hemosiderin) is found in the pigment of the liver, spleen and kidneys. The erythrocyte count is frequently below 2,000,000, yet the patients may be going about and not bed fast in some cases. Megaloblasts are always present, some times in great numbers. Poikilocytosis is always present. Normoblasts are found less frequently than the megaloblasts. There usually is a moderate lymphocytosis. A few myelocytes may be found. The hemoglobin may be only 20% and the erythrocytes only 30% of the normal.

Chlorosis a form. of primary anemia, with a marked reduction of hemoglobin, with a slight numerical variation of normal erythrocytes; although they may be reduced to 600,000 in some cases. The color index is low. There is no leukocytosis. The white blood cells consist of four chief forms, the lymphocytes, small and large, and the polymorphonuclears, many of which contain coarse granules, and the eosinophiles.

Leukemia.—A great number of leukocytes, associated with anemia; the more marked the change in the erythrocytes, the more severe the disease. There may be found 200,000 or more leukocytes.

Lymphatic leukemia.—Indicated by glandular enlargements, erythrocytes reduced about one half, myelocytes absent and normoblasts rarely found; but there may be a lymphocytosis predominating in acute lymphatic leukemia but rarely exceeding 125,000. Hodgkin's disease is marked by the grandular enlargement, but shows a negative blood picture.

Spleenomyelogenous Leukemia or myeloid leukemia.—Differs from leukocytosis by depending upon the large numbers, or proportions of myelocytes, neutrophilic and eosinophilic myelocytes. The blood is of a milky buff color. The marrow is replaced by a yellow and hyaloid material. The spleen becomes very much enlarged and may weigh 10 or more pounds. The leukocyte count is 2 to 5,000, of which 30 to 50% are neutrophilic myelocytes and about an equal number of polymorphonuclears; the lymphocytes average 2 to 5%. Normal eosinophilic myelocytes and large mononuclears make up the remaining percentage. Megaloblasts are rarely found. The erythrocyte count is from 2 to 3,000,000.

Some diseases call forth an extra number of leukocytes, as pneumonia and septic diseases.—Leukocytosis (polymorphonuclears). Such diseases do not show an antitoxic or anti-

bacterial serum on recovery; the phagocytes are believed to be the principal agents in overcoming the disease by ingesting or destroying the invading organisms, or by stimulating the vital functions or processes. Other processes as injury to the lymphatic tissue, spleen, skin, etc., call forth an increased number of leukocytes.

The laking of the blood occurs when the serum of the animal is injected into the circulation of another animal. Blood may be deficient in hemoglobin which is carried by the red cells—Anemia. If the cells are reduced in numbers only, it is secondary anemia. If certain pathological forms appear it is pernicious anemia. The blood has natural antibacterial and antitoxic powers which are overcome by a number of causes as exhaustion, chilling, exposure, malnutrition, excesses, et al. It may be increased by the successful recovery, after having had the disease.

THE OPSONIC INDEX

The opsonic power or index indicates the patient's capacity to resist a certain disease as compared to that of a healthy person.

To obtain the index, emulsify completely a small loop full of the young agar culture of the organism to be studied in 10 drops of normal salt solution, which contains 1% sodium citrate to prevent coagulation of the blood, in a sterile watch glass. Puncture the patient's ear or finger and draw the blood to a marked point of the pipette, draw in a little air to break the column; then draw up the bacterial emulsion to the same mark to which the blood had been drawn, mix by repeated drawing up and ejecting of the mixture into the watch glass, until mixed. Draw mixture into the pipette until it reaches a 5 cm. mark, seal both ends in the flame and incubate at 37 degrees C. for 12 minutes. The pipettes used are capillary tubes made by drawing glass tubing out in the flame.

Prepare a similar pipette but use your own blood, or that from a normal person, and incubate 12 minutes.

After incubation, file off the ends of the pipette, eject the contents on slides, mix thoroughly to secure a thin film, air dry and stain by one of the various blood stains, or alcohol may be added to the air dried slide, the alcohol ignited, this fixes the blood and bacteria to the slide, stain with carbol fuchsin for 15 seconds, wash with water and examine. Count the phagocitized bacteria (that is count the number of bacteria which have been engulfed by the leukocytes) in a given number of polymorphonuclears. Calculate the average number of phagocitized bacteria to the cell. Repeat this process with the other pipettes of blood and bacteria. Then divide the percentage average of the patient's blood with that given by the normal blood and this gives the opsonic index. Example: If the patient's blood has 8 out of 50 cells which have engulfed bacteria, and the normal blood has but 4 out of 50, then the patient's index is 2 or twice the normal. There are two phazes to the opsonic index—the positive and the negative phazes. The negative phaze is seen when a suitable dose of bacterial vaccines is given which will cause a fall in the amount of opsonins present in the blood stream; it may last from a few hours to several weeks and it is followed by a gradual rise in opsonins which produce the positive phaze.

Remember that the leukocytes contain granules, or

nuclei; and that the erythrocytes contain no nucleus. That basic stains are the blues and browns and that the acid or acidophilic stains are the reds and yellows.

Cells containing granules are:	Cells devoid of granules are:
Polymorpho leukocytes.	Lymphocytes.
Polymorphonuclear leukocytes.	Leukocytes.
Neutrophilic myelocytes.	
Myelocytes, large mast cells.	
Eosinophiles.	
Basophiles.	

The erythrocytes of the human measure 1-3200 of an inch in diameter; of the dog 1-3542 of an inch; those of a cat 1-4404; hog 1-4230; horse 1-46000; sheep 1-6355; musk ox 1-12325. They are all non-nucleated and appear as rounded, or on a side view as biconcave disks. Those of birds, fish and reptiles as oval and contain a nucleus.

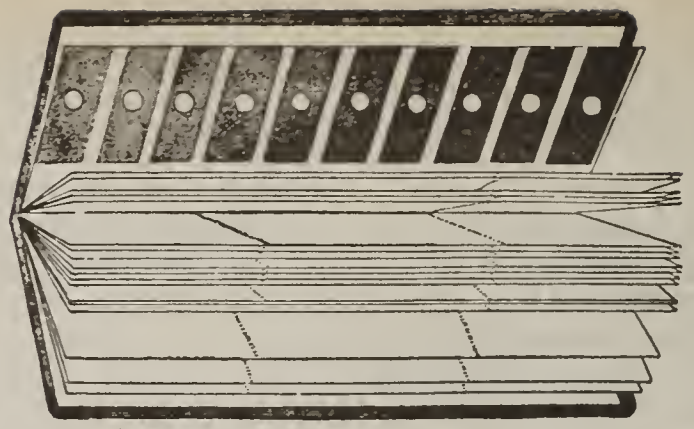
Bleeding is stopped by injections of normal horse, rabbit or human serums, or anti-diphtheric serum. Warm compresses at the site of the wound, tourniquets, etc. Dog and cow serums are poisonous to man. Calcium salts aid coagulation.

Coagulation is prevented by taking citric acid in 3 gram doses in water, every three hours, or saturated solutions of citric acid, or sodium citrate 1.5 grams, sodium chloride 5 grams and water 100 mils.

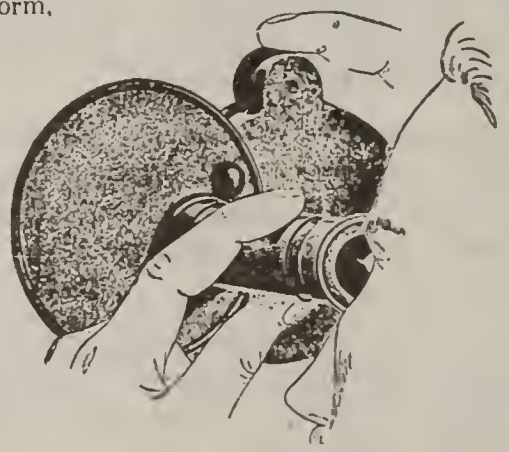
Hemoglobin tests:

1. Hemoglobinometer.
2. Hamoglobin scale.
3. Conjunctiva test.
4. Finger nail test.

Wounds made during life and after death may be distinguished by the large numbers of leukocytes in wounds made during life, while wounds made after death contain few or none. This test is not accurate when the wounds involve large vessels or a long time after putrefaction has set in.



HAEMAGLOBINOMETER, TALQUIST'S, is a simple bedside test adapted to the use of every physician on account of the ease and accuracy with which the test may be made, and its inexpensiveness. It consists of 50 pages of strips of absorbent paper with scale attached showing tints of haemoglobin from 10 per cent and 100 per cent, bound in book form.



HAEMAGLOBINOMETER, DARE'S, deals with the undiluted blood which is drawn by capillarity into a very thin chamber of definite thickness and which is compared with a colored prism by modified light from a candle [illegible]

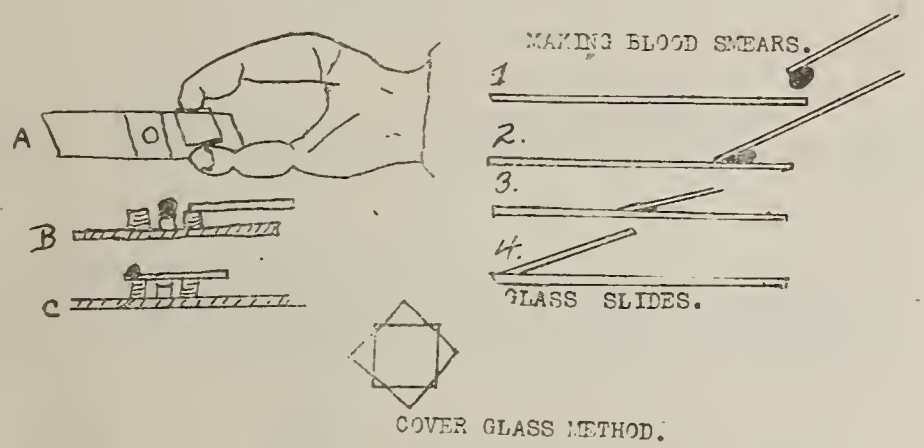
HAEMAGLOBINOMETER, SAHLI, for determining the percentage of haemoglobin in the blood. A measured amount of blood diluted with distilled water is placed in a graduated tube until it is of the same color as a tinted tube provided, when the percentage is read off on the scale. Accurate to within five or ten per cent.

EXERCISE.

1. Wash the finger tip, paint with iodine solution.

2. Sterilize a new pen point (one prong of which has been broken off) by heating until red hot, cool and.

3. Puncture with a quick stab and make 6 films on clean slides (slides which have been prepared in the sulphuric acid cleansing fluid, etc., and have been flamed). Do not use pressure or squeezing to obtain the blood, as this causes a dilution of the blood with serum from the surrounding tissues. make the films by touching the end of one side to the drop of blood, then to the end of another side and push over the surface of the second slide with a steady motion and without pressure.



COVER GLASS METHOD.

4. Air dry.

5. Fix one slide by adding methyl alcohol and setting on fire at once.

6. To this slide add a 1% aqueuos solution of eosin for 20 seconds, wash in water, then apply Loeffler's stain for 3 minutes, wash in water, dry and examine.

7. With another slide, fix in the flame by passing the slide held in the hand, film side up, through the flame 3 times at the rate of a pendulum, and stain as directed in No. 6.

8. Fix another slide in the flame, when it has cooled, add the eosinate of methylene blue. This is a double stain, that is staining both the red and white cells at the same time. Add sufficient of this stain to cover the film, permt it to stain exactly 60 seconds; at the end of 60 seconds add to the slide two times as much water as there is stain on the slide; do not

add so much water that it will overflow from the slide. Add sufficient water that a metallic scum forms. Permit this mixture of stain and water to remain on the slide 2 minutes. Then wash the slide in water, dry and examine.

Use great care, the correct time, and remember that the films of blood on the slides must be one cell in thickness.

Draw the leukocytes, and name each kind.

Test for Hemin crystals. Place one large drop of blood on a clean slide, then add a drop of 20% glacial acetic acid, evaporate in the air; then add a drop of sodium chloride solution, and warm, examine for the brownish red crystals.

PROTHROMBINS ← ANTITHROMBIN

CALCIUM → THROMBIN → FIBRIN CLOT

KINASE? ↗ FIBROGEN ↗

OR

HOWELL-TISSUE JUICES NEUTRALIZE ANTITHROMBIN (IN NORMAL BLOOD)

OR

ANTITHROMBIN ← THROMBOPLASTIN

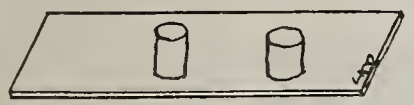
↔

PROTHROMBIN → THROMBIN (FROM LIVER) → CLOT

CALCIUM ↗ FIBROGEN (FIBRIN LIKE ↗ BODY REMOVED BY COAGULATION)

THEORIES OF BLOOD COAGULATION.

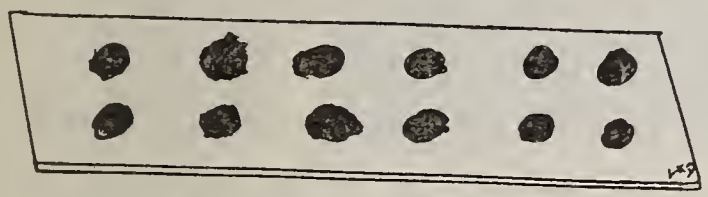
BREAK OFF A SMALL PIECE OF THE CAPILLARY TUBE, (FILLED WITH BLOOD) EVERY 3 MINUTES. NOTE TIME OF COAGULATION.



SMALL GLASS TUBES CEMENTED ON GLASS SLIDE, BY TOUCHING CLOT WITH END OF BROOM STRAW, COAGULATION TIME IS DETERMINED.

A LARGE DROP OF BLOOD PLACED ON A CLEAN SLIDE.

COMPLETE. INCOMPLETE CLOT.



12 DROPS OF BLOOD ON A CLEAN SLIDE, DRAW THROUGH ONE DROP EACH MINUTE, A CLEAN BROOM STRAW, WHEN THE DROP ADHERES AND FIBRIN THREADS PREVENT THE STRAW FROM BEING DRAWN THROUGH THE CLOT CLEAN, IS THE COAGULATING TIME.

DETERMINING TIME OF COAGULATION.

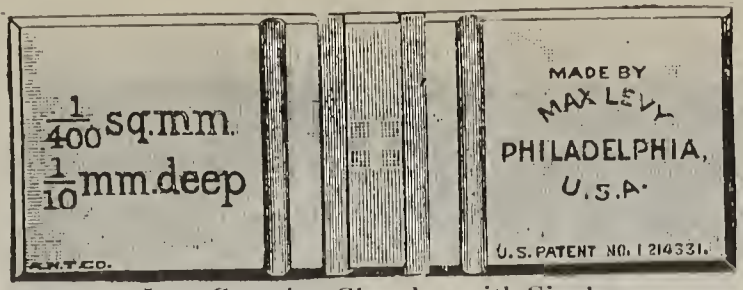
The apparatus consists of a ruled chamber, ruled according to Thoma, Zeiss, or other reliable rulings, pipettes marked 1-101 for the erythocytes, and one reading 1-11 for the leukocytes.

Blood from a punctured part obtained as directed under the differential count is drawn up in the erythrocyte pipette to the mark 0.5, then filled to the mark 101 with Hayem's solution (see stain formulas) well shaken, several drops blown out, a drop placed on the counting chamber and the special cover glass adjusted. The preparation is permitted to stand for several minutes, so that the cells will gravitate to the bottom and rest on the surface of the counting chamber.

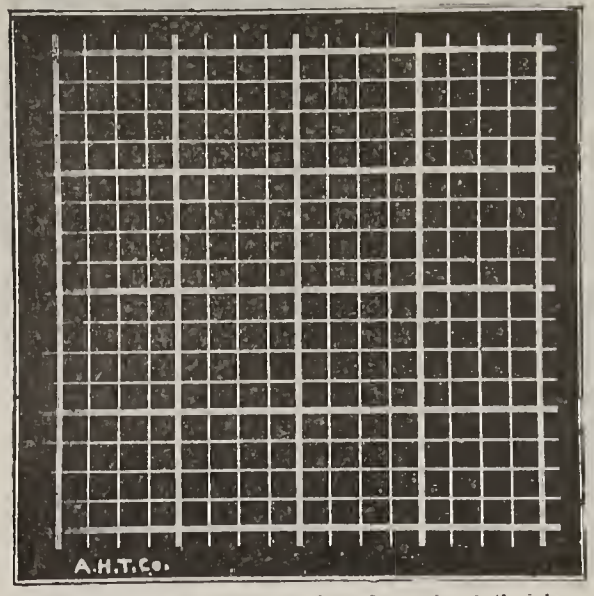
100 small squares are counted, the number of cells in each noted, and the total multiplied by 8,000 to get the total count per cubic millimeter. This figure 8,000 is derived from the fact that there are 400 small squares to the square mm. that the chamber is 1-10 mm. deep and that the dilution of the blood is 1 to 200, hence 200x10x4 equal 8,000.

The leukocyte estimation is made in the same way, using the leukocyte pipette, drawing the blood to the 0.5 mark, and the diluting fluid drawn up to the 11 mark, shaken and the first drops from the pipette discarded, a drop placed in the counting chamber, the cover carefully placed, and all the large cells counted, or 9 large cells counted. Divide the total number of leukocytes counted by the number of cells counted to get the number of leukocytes in 1 square millimeter. Then multiply by 100 (the depth of the cell in the counting chamber is 0.1 mm. and the dilution is 1 in 10) this will give the number of leukocytes in 1 cmm. of blood. Or the pipette used for the erythrocytes may be used for couning the leukocytes, but in this case the dilution is 1 in 100; then the average number of leukocytes counted time 1,000, would give the total number in 1 cmm. of blood.

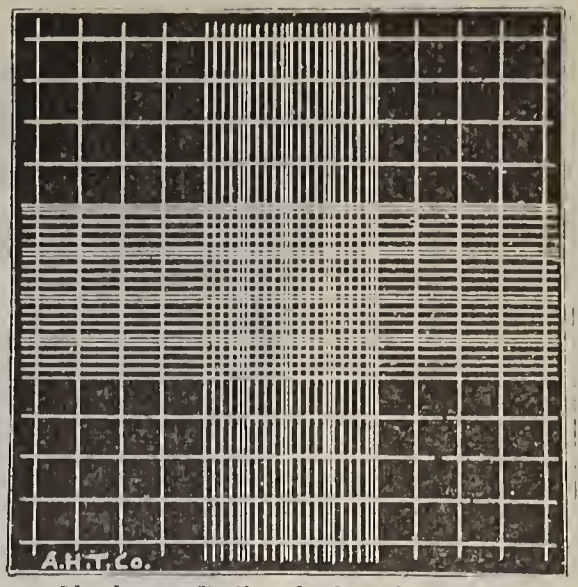
Vertical longitudinal section of slide, with cover glass in position, showing new method of construction



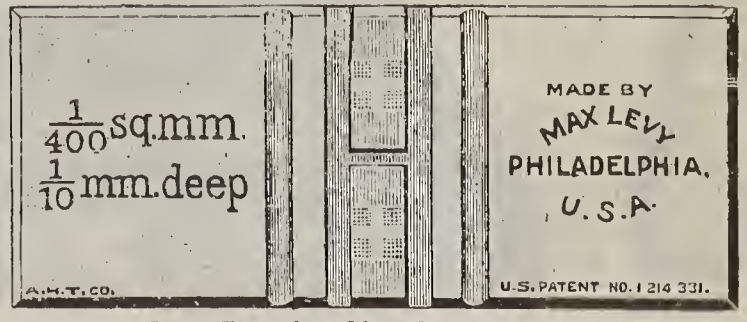
Levy Counting Chamber with Single Neubauer Ruling



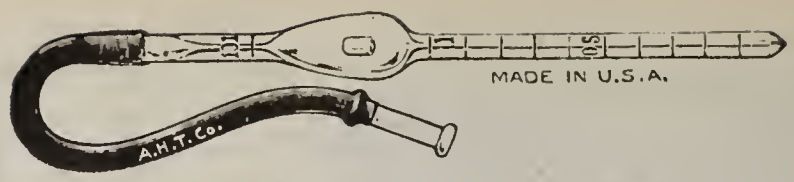
Fuchs-Rosenthal Ruling for spinal fluids



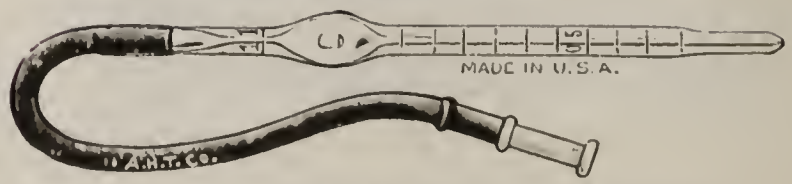
Neubauer Ruling for blood counting



Levy Counting Chamber with Double Neubauer Ruling



Mixing Pipette for red corpuscles.



Mixing Pipette for white corpuscles

COUNTING THE NUMBER OF CELLS IN SPINAL FLUID

The total count is made in the same way as for the blood. A diluting fluid of a very dilute solution of methyl violet is used. The differential count is made from centrifuged fluid and stained with an aqueous solution of methylene blue. There are normally about three lymphocytes in 8 mils of fluid and no morphological elements present. Micro-organisms and protozoa occur according to their respective diseases.

EXAMINATION FOR THE MALARIAL PARASITE

Fresh specimens may be examined by pressing a small drop of blood between a cover glass and the slide and using the oil immersion lens.

The dried blood films may be stained with the ordinary blood stains.

THE DIFFERENTIAL BLOOD COUNT

A differential count consists of counting the number and kinds of white blood cells.

A NORMAL DIFFERENTIAL COUNT

Polymorphonuclears	60	to	75%	or about	5,000	per cmm.	of blood
Small lymphocytes	15	to	30%	or about	1,500	per cmm.	of blood
Large lymphocytes	3	to	10%	or about	500	per cmm.	of blood
Transitionals	2	to	4%	or about	200	per cmm.	of blood
Large mononuclears	1	to	2%	or about	100	per cmm.	of blood
Eosinophiles	1	to	4%	or about	200	per cmm.	of blood
Myelocytes	½	to	1%	or about	40	per cmm.	of blood
Mast cells (Polynuclear basophiles)	¼	to	1%	or about	25	per cmm.	of blood

The lobe of the ear, ball of the finger or in small children the ball of a toe is cleansed with cotton moistened with Tr. Iodine; after the cleansed part has dried, it is punctured with a new pen point, one point of which has been broken off, the remaining point heated until red hot, cooled and the puncture is made with a quick stab, sufficiently deep to cause the blood to flow freely, so that squeezing is not necessary, as this will cause an exudation of serum and dilute blood, thus rendering the count inaccurate. The pen point once used is thrown away, a new one being used for each patient.

The films of blood are obtained by touching the end of a previously cleansed slide, to the drop of blood on the punctured part; the slide is then touched to the end of another slide and

with a rapid motion, without pressure, is pushed over the surface of the slide; in this way a film of blood is obtained, one cell in thickness which is essential for good counting. At least four such slides are made.

The films are stained by any reliable stain, one of which is the eosinate of methylene blue, (formula given under stains) which without any preliminary preparation of the slide, is added in sufficient quantities to flood the filmed portion; at the end of one minute two times as much water as stain on the slide is added; this counter staining is carried on for two minutes. At the end of this time the slide is washed with water, blotted lightly with a lintless blotter and is ready for examination.

The mirror of the microscope is properly adjusted, the stained slide placed on the stage, the barrel of the microscope with the 4 mm. lens is racked down until almost touching the slide, the barrel by means of the coarse adjustment is racked upwards until focus is obtained and the view perfected by the fine adjustment.

The count is made by beginning at the upper left hand corner of the slide, and noting on a ruled paper, (see cut) in the different spaces provided, the various forms of the Leukocytes, which appear as large cells, usually staining blue and nucleated, while the Erythrocytes are smaller, non-nucleated and stain red or pinkish. After all the Leukocytes in the field are counted the slide is moved to the left until a new field is seen, the Leukocytes counted, the slide is again moved and so on until the end of the slide is seen. The slide is then moved up a sufficient distance so that the lower cell of the last field counted has just disappeared. The count is continued by moving the slide from left to right and so on until at least 500 Leukocytes have been counted. From the ruled blank the percentage of the different cells are calculated. As an example, if out of the 500 leukocytes counted, 20 were eosinophiles, then 20 times 100 divided by 500, or the total number of cells counted would equal 4% of eosinophiles.

DIPHTHERIA

A specific disease characterized by clinical symptoms arising from corresponding toxins, produced at the site of of the membrane or wound, which causes a false membrane at the site of the infection.

The Bacillus Diphtheriae produces a soluble toxin (the toxin which during the life of the organism diffuses out of their bodies and into the surrounding tissues). This toxin is filterable and will combine with antitoxin in the test tube. The lymphatics absorb some of the toxin.

The bacillus was discovered by Klebs in 1882 and the description was completed by Loeffler in 1884, hence the name Klebs-Loeffler bacillus. Roux and Yersin established the specific relationship of the bacillus to the disease and proved by Koch's laws.

This toxin is destroyed in five minutes by boiling. It is weakened by very low temperatures and is killed in a few hours by sunlight. If kept in the dark and in cold storage it will live for over two years. The toxin is destroyed by small amounts of acetic acid, and the toxin bodies are precipitated by alcohol, or calcium chloride, and if these are injected into animals the symptoms of the disease are produced.

The bacillus is non-motile and non-sporing. It is about 1 by 6 microns or 6 times as long as broad, one end somewhat bulbed, and presents a beaded or granular appearance, so that the microscopical diagnosis is easy. It is readily grown upon blood serum media. It is destroyed by a 1 in 1,000 bichloride solution, but may remain virulent when dried for over 6 months. It is found in the throat two or more years after infection—Diphtheria carriers—those who do not have the disease but are able to infect others. About 1 to 3% of all healthy persons are diphtheria carriers, they have the bacilli in their throats but shown no signs of the disease.

The tonsils act as a filtering agent, for all the air drawn into the lungs passes over them and the bacteria are taken out of the air by sticking to the excretions of the tonsils. The tonsils are the seat of manufacture of an antitoxin against diphtheria. They should never be removed; but if they are diseased, only the diseased portion should be removed.

"Is a Child's Life Worth Six Dollars," is a heading in the Ohio Public Health Journal. It states that in Salem, Ohio, during 1890 to 1899 there were 162 cases of diphtheria with 30% deaths. In 1900 the city began furnishing free

antitoxin and from 1900 to 1910 there were 126 cases, and only one death; and in this case the antitoxin was not given until the third day. Salem would have had many more deaths if it had not been for the free antitoxin, which cost $220, or an average of $6 for each life saved.

The disease is transmitted by kissing, toys, dust, clothing, animals, et al.

Abrasions of the throat are easily infected, and in some cases infections of the nose, eyes, ears and skin are seen. The incubation period is about 48 hours.

Streptococcus is usually associated with the disease.

A lymphocytosis and a leukocytosis occur but no agglutination.

As a prophylaxis 1,000 to 10,000 units and as a curative 10,000 to 50,000 units of Serum Antidiphthericum are given. Antitoxin has reduced diphtheria mortality nearly 70 to 90%. This antitoxin will give an immunity lasting about three weeks.

Post-diphtheric paralysis usually results from not sufficient antitoxin being used; this paralysis does not come as many suppose from the antitoxin but from the toxin of the bacillus which has not been neutralized by giving sufficient antitoxin. Too much antitoxin can not be given, it is better to use too much than not enough. Paralysis, heart failures, throat and muscle paralysis many times follow the recovery from the disease. Unless given too late, antitoxin usually cures these after effects or dregs.

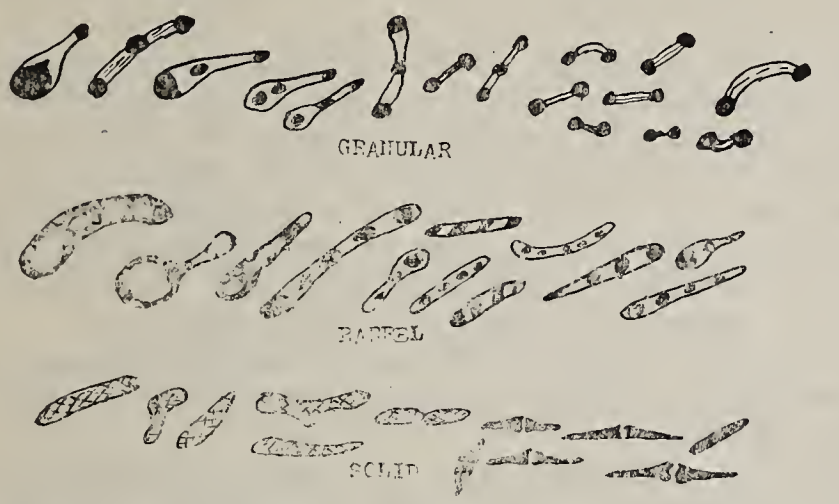
The antitoxin contains the antibodies (see pages on antitoxins) which are obtained from suitable animals as the horse, by injecting ascending doses of the toxin, which calls forth a corresponding increase of antibodies in the blood of the animal—these antibodies are the defensive substances.

Classification of Bacillus Diphtheriae.—Westbrook's.

1. Granular forms occur as distinct spherical, ovoid, or markedly rounded granules. These granules show metachromatism and are fairly uniform, and are found at one or both ends of the bacilli. The protoplasm which is not in the granules stains less intensely. This type is rarely found in clinical cases.

2. Barrel forms are distinguished by their irregular staining, presenting somewhat of a barrel appearance, and as if the segments of protoplasm had been removed. The darker portions vary in staining and the segments or the

darker portions number from 3 to 9. This type is usually found in "carriers."

3. Solid type—when the bacilli are of a uniform, solid form, size and staining. Some may appear as diplococci.

WESTBROOK'S TYPES OF BACILLUS DIPTHERIAE.

False diphtheria bacillus occurs in false membranes in the throat, and does not kill guinea pigs, does not produce toxins, and does not cause the production of an antiserum. It grows only on the surface of the culture media and does not turn litmus agar red; in fact it is just opposite to the true diphtheria bacillus in the mode of growth, etc.

Loeffler dried diphtheria bacillus on silk threads and after four weeks found the bacillus viable. It lives on dried membranes 16 weeks or longer. Membranes inoculated and kept in a dark room at room temperatures will remain viable for months. Abel found the bacillus on a toy with which a diphtheria patient had played six months before.

In the United States the mortality from diphtheria before using antitoxin was 39%; after the use of antitoxin had become prevalent the mortality dropped to 14%. For five years from 1915, Chile has had a death rate of 6.8 per 100,000; 7 in 100,000 in New Zealand and the Netherlands; 22.6 in Prussia, 25.9 in Austria and 40.1 in Serbia.

Rolleston in 12 years study of 2600 cases found less than 20 cases or 1% in the first year of life. He found 668 cases, or 25.6% with diphtheria of the membranes of the nose, 13 cases of nose and throat diphtheria. Cutaneous cases were 15%. Paralysis resulted in 6 of the 20 cases and 5 were fatal;

in the first year of life, 9 died of cardiac failure, two of congential syphilis and 2 of broncho-pneumonia.

Thirty-two per cent, of the recoveries in children act as carriers. Cultures must be taken from nose and throat, and nose and throat combined and three negative cultures taken three days apart before the patient is discharged.

Thirty-five per cent. of the population are susceptible to diphtheria; about 65% show a natural immunity.

In an epidemic in a children's home, 65 persons contracted diphtheria; one of the girls in the home acquired the disease; cats had the run of the houses, and carried the diphtheria bacillus from this one patient to the other girls and to the boys' house situated some distance away. Cultures from the cat's throat showed the bacilli in great numbers.

The bacillus has been found in healthy throats, pillows and bedclothes, drinking glasses, nipples or nursing bottles and other places.

The most susceptible age is between 1 and 5 years. Immunity is obtained by having the disease; this immunity will last from one month to several years. This immunity as well as that produced by antitoxin will vary greatly in different individuals, and is very brief in children.

It has been found that about 80% of the new born babe's blood serum will contain sufficient antitoxin to make them non-susceptible to diphtheria, and that 50 to 60% of children and 90% of the adults show this immunity; this was determined by:

THE SHICK DIPHTHERIA TEST

The skin of the flexor surface of the forearm is cleansed with soap and water, dried of 0.2 mil of normal sterile salt solution in which has been dissolved 1-50 the M. L. D. of diphtheria toxin is injected, just under the skin. If the infection is properly performed a white bleb like elevation is seen at once and persists for several minutes; it is distinctly studded with little pits, corresponding to the hair follicles. After 24 hours, if there is not sufficient antitoxin in the patient's body to protect against the disease, a positive reaction appears; this is characterized by a constantly increasing circumscribed area of redness (halo), of from 10 to 25 mm. in diameter. After it reaches the maximum in 48 hours and persists for about one week it fades, showing a brown pigmentation with superficial scaling and a central infiltration. Positive reactions indicate that there is less than 1-30

unit of antitoxin in each 1 mil of blood serum. Such persons exhibiting the positive reaction are very susceptible to diphtheria. The intensity of the reaction will vary in different patients, a well marked redness indicates a complete absence of diphtheria antitoxin; a faint reaction indicates the presence of a small amount; and a negative reaction indicates the presence of sufficient antitoxin in the patient's blood serum to protect against the disease. Control injections of sterile bouillon should be made near the site of the toxin injection.

The toxin taken by filtration from a three weeks old culture and injected in doses of 0.001 mil will kill guinea pigs within 24 hours.

The toxin is composed of two parts, the toxin and toxone.

CULTURES AND STAINING

Cultures are taken by means of the swab stick, which is about 12 to 15 cm. long and of thin round pieces of wood or wire with cotton tightly wrapped around one end, enclosed in a cotton plugged test tube, with the cotton wrapped end resting on the bottom of the test tube, and sterilized. This serilized swab is removed from the test tube, at the bedside, and carefully rubbed over the inflamed area as of the pharnyx and tonsils and a tube of Loeffler's medium inoculated, and several films are made on glass slides with the swab, the swab returned to the test tube and later sterilized. The cultures are incubated 24 hours at 37 degrees C. The culture medium is made according to the formula of Loeffler's blood serum agar, and it is adjusted to 1% acid with phenolphthalein but alkaline to litmus. Three parts of calf or sheep blood serum is added to a 1% glucose bouillon. This makes a very good liquid medium. The cultures appear on solid media as small opaque gray centered colonies, growing on and just beneath the surface. The colonies are granular, irregular or ragged edged, at first moist but later dry. The bacillus will not liquefy gelatin. It produces an abundant growth in milk, with the granular virulent types and no fermentation with lactose. In dextrose bouillon it produces an acid reaction giving an acid reaction without curdling. It will not be fermented by the solid type.

The bacillus will live on agar media for six or more months, in dextrose broth one year or more, but of course the virulence is lessened. They are killed by 55 degrees C. heat for 45 minutes.

The diphtheroid bacillus resembles the diphtheria bacillus, but is less virulent and is shorter and wider. It shows no granules with Neisser's stain, and produces no acid in dextrose bouillon and is non-virulent for guinea pigs; while with the true diphtheria bacillus guinea pigs die in two to three days after injections of young bouillon cultures, developing nephritis, paralysis, etc. Koch's laws can be fulfilled with the true diphtheria bacillus.

Animals as cats and dogs are very susceptible to diphtheria, mice and rabbits are less susceptible. Pigeons are very susceptible.

In the disease a leukocytosis is found, proportionate to the exudate present; the leukocytosis may be over 100,000, of which about 80% are neutrophiles consisting chiefly of polymorphonuclears, a small number of neutrophilic myelocytes—if found in large numbers it indicates unfavorable prognosis. Injections of antitoxins cause a drop in the leukocyte numbers but a slight increase of the erythrocytes. Hemoglobin is decreased about 10%. Films from the throat show cocci, pus, degenerated epithelial cells as well as the bacillus. Albuminuria is found in 5% of the cases and usually disappears on convalescence.

EXERCISE

1. Make films on slides from throat, swab or culture.
2. Stain with Gram stain. (Gram positive.)
3. Stain with Neisser's double stain, solution No. 1 for three minutes, wash with water, and stain with solution No. 2 for three seconds, wash with water.

SMALL POX

Small pox is an acute contagious disease, characterized by a sudden onset, a severe period of invasion and followed by a recurrence of fever and an eruption, passing through three stages: Vesicle, pustule and scab.

The disease lasts from 4 to 5 weeks and it is transmitted by direct contact, through the excretions, blood, skin, lesions, scales or crusts, etc., of the patient.

The great plague at Athens during the Peloponnesian War was small pox. Plagues of small pox were known in Bible times and prior to the 12th century in China, but it prevailed in India at an earlier date. The disease was first described by an Arabian physician, Rhazes, who died in 932 A. D. It was probably known in Europe prior to the 6th century and was introduced into Mexico by the Spaniards in 1520, destroying 3,500,000 people and in many places wiping out whole tribes. It was first seen in Ireland in 1707 and 18,000 out of 50,000 people died. From the 15th to the 17th century it was universal and it is estimated that 4,000 deaths per million occurred; and other authorities as Dr. Farr say: "One twelfth of the total mortality was due to small pox." In 1717 Lady Morley Montague, wife of the British Ambassador to Turkey, wrote to England describing the "conferred" immunity, by taking the virus from a diseased person and inoculating a well person. This method continued in Europe for many years.

In the early part of the 17th century observers noticed that in dairy districts, persons became infected with a localized skin affection known as "Cow pox" acquired when milking or handling cattle affected with sores on the udders, and that these people escaped small pox. Dr. Fewster of Glouchester in 1765, sent a paper to the London Medical Society, treating on this infection. In 1774, a farmer, Benjamin Jetsy of Dorcestshire, vaccinated his two sons and wife with this cow pox virus. Jenner, a student of medicine under the famous John Hunter of London, began his practice near Berkley, where he learned of Jetsy's vaccination. After experimenting he finally inoculated a boy, James Phillips, May 14, 1796, with the virus from the hand of an infected milk maid. A successful vaccination resulted, and within a few weeks, Jenner inoculated the boy with virus taken from a small pox patient, without the boy taking small pox. He reported this result to the Royal Society, June, 1798. This method was tried in many hospitals with great success, but later, vaccina-

tion was prohibited in England, on account of some people who thought that the characteristics of the cow, such as horns, tails, etc., would be transferred to the vaccinated person; and even to this day, vaccination is not compulsory in England.

In 1802, Dr. Woodville, inoculated 7,500 patients; and about one-half of these were later inoculated with small pox virus without contracting the disease. The first recorded vaccination in this country was in 1802 by Dr. Watterhouse of Boston, who used the early method—arm to arm; that is, a healthy child was vaccinated, and about the 8th day, when the vesicle was ripe, part of the clear serum was dried on ivory points. In this country, as well as in Turkey, India and China, the scab method was first used; that is, when the scab of a vaccinated person dropped off, it was carefully wrapped in beeswax until needed, then the arm was scarified, and the scab rubbed on. Many diseases were carried in this way and often fatal cases of septicemia resulted.

In 1842 Negri first used a calf for the propagation of the virus. This method was introduced into this country by Dr. Mart, of Boston, in 1870. Scabs from the calf were used, then goose quills dipped in the sores and dried, and later ivory points; but the great objection to this method was that it always carried infection.

Dr. Copeman in 1898 used glycerin to increase the bulk and to preserve the purity. Today only the glycerinated virus in capillary tubes is permitted to be used in the United States. This prevents all outside contamination.

Vaccination with pure virus (no contamination in the manufacturing) and when properly applied will produce a small typical umbulated vesicle and not the large, deep ulcers usually formed; these are infections and not vaccination.

The virus from cow pox is official in the United States as Virus Vaccinicum (see your U. S. P.), and must be preserved between 4.5 degrees to 15 degrees C. and away from the light, as it loses its virulence rapidly unless kept under these conditions. Dr. Elgin experimented with the viability of vaccine virus and found that a temperature of 60 degrees C. for 5 minutes kills the vaccine, 5 minutes at 55 degrees C. weakens it and 37 degrees C. for 3 to 4 days kills it. It will remain active for 3 months at 10 degrees C. Vaccine on Petri dishes showed at 58 degrees C., 69 colonies of bacterial growth, 3 days at 37 degrees C. 1 colony and 5 weeks at 21 degrees C. none, and 10 weeks at 12 degrees C. none; and after the vaccine was 4 years old and then cultured,

staphylococci was isolated from it and these were pathogenic, killing rabbits.

Vaccination should take place immediately after birth, or at least before the first six months of the child's life, and revaccination every 5 to 6 years. A "take" will occur if good viable vaccine is used and if properly applied in 99.0% of persons who have never been vaccinated. Immune persons will also have a take, or in other words, a person may be successfully revaccinated any time if the vaccine is viable and properly applied.

In vaccination the outer aspect of the upper arm should be cleansed with soap and water, then with alcohol, or gasoline, but not with antiseptics; expel the vaccine from the capillary tube on the cleansed spot, grasp arm so that the skin will be stretched and scarify the cuticle with a sterile needle or ½ of a new sterile pen point, or it may be rotated; care must be taken not to draw blood or the virus will be washed away; rub in well and permit to dry in the air, not sunlight, or it may kill the virus. No coverings are necessary. When pustules form and are ready to break, moist dressing may be applied and renewed every two hours.

Dr. Balmis, a Spanish physician, was sent by the Spanish king in 1803 to all his colonies to vaccinate his subjects. He was nearly 3 years on this expedition. Holland has sent similar expeditions to her colonies.

Japan has reduced the mortality rate from small pox in 1887, from 9,976 in 40,000 cases to 110 out of 4,830 cases in 1914 by the use of vaccine virus, and has a very strict compulsory vaccination law.

In the war of 1870-71, the French army had 25,000 deaths from small pox.

In the war of 1915-19, there were only 12 cases of small pox and 1 death. This is the first war in history which has not been accompanied with epidemics of small pox.

TETANUS—LOCK JAW

This is an acute infectious disease characterized by rigid spasmodic contractions of the muscles and caused by the Bacillus Tetanus, which is a sporing, flagellated anaerobe—discovered by Nicholaier in 1884, and cultured in 1889 by Kitasato. The toxin was discovered by Vaillard and Vincent and the antitoxin by Behring and Kitasato.

On account of the spore forming in one end of the bacillus, it appears like a tack or drum stick. The bacillus is found in street air, manure (and passing through, after the animal has eaten infected food), impure gelatin (which has been injected to stop bleeding) and may be found in all kinds of wounds. It is normally in intestines of horses and other animals which are not very susceptible. The spores may live for 10 or more years, but are killed when in a dry condition in 6 days by direct sunlight; they are killed by a 5% phenol in 15 hours.

The incubation period in 50% of tetanus cases is from 4 to 10 days and the acute form of the disease lasts from 1 to 10 days, and the subacute 10 to 20 days. Tetanus of the head causes a more rapid death. The spores are the infective agent and the bacillus cannot live in the air. The spores belong to one of the most commonly found pathogenic organisms. When a wound occurs, and is contaminated with spores, as in the case of a bullet wound, passing through contaminated clothing, the spores may be carried into the wound, which may furnish all the requirements of bacterial growth—especially if the spores have lodged at the bottom of the wound and are covered over with exudations—the wound having healed on the surface, and the supply of oxygen being shut off. The spores will grow rapidly and produce the bacillus, which excretes the toxin. This is carried to and affects the nervous system along axis, cylinders, and ganglion and lymphatics, thus influencing the muscles, causing their contractions and the ricersardonic grin, when the muscles of the face and neck are contracted and give to the patient's face a peculiar expression. The longer this is delayed, the better the prognosis. A general tightening of the muscles follows, aches, pain and lassitude with the patient conscious until the last. Sometimes the abdominal muscles tighten so much that the abdominal wall is bursted. The disease has a slight leukocytosis averaging 13,000 and the hemoglobin is about 75%.

Phenol is used as a prophylactic in Italy; in the United States the Serum Antitetanicum is used, repeated every 10

days. There is no certain cure after the symptoms have developed; and there is little hope for recovery. Ten to 20,000 units are injected intravenously or 3 to 5,000 units intraspinally and repeated according to the case. Intravascular injections are more rapid in effect, than cutaneous, which takes about 40 hours for action. Antitetanic serum should be also injected into the wound, which usually has healed (especially on the surface). Magnesium sulphate spinal injections have been tried along with many other things, but without much success. Chloral and anesthetics are used to control the convulsions, and also are given at the time of injections so that convulsions will not be produced, for shock, sharp noises, talking, etc., may start the patient into convulsions. Antitoxin dusting powder composed of the dried serum and antiformin equal parts has been used very successfully. Antitoxin has reduced the mortality 20%. 90% of all cases are fatal.

In the last war all the wounded American soldiers received as soon as possible 500 to 1,500 units and 6 hours later another injection of 1,500 units; this practically eliminated tetanus among our soldiers.

All wounds must be kept clean and open. A wound which has been opened so that proper cleansing may take place and which heals from the bottom, rarely produces tetanus.

The bacillus in broth cultures will produce spores at 37 degrees C. in 24 to 30 hours. The spore is spherical and 2 to 3 times the diameter of the bacillus and is formed in one end of the bacillus. It is Gram positive and is motile. It grows slowly on gelatin media and liquefies it and also blood serum agar. In stab cultures it resembles a fir tree and produces a small amount of gas, and will coagulate milk with an acid production.

The bacillus is isolated by heating pus or mixed cultures in live steam for one hour at 80 degrees C. This will kill nearly all other organisms, but the animal inoculation method is more reliable.

A unit is 1,000 times the M. L. D. for a 350 gram guinea pig.

TYPHOID FEVER

This disease attacks by preference the young and middle aged, and "causes about 400,000 persons to be incapacitated, and 30,000 deaths annually in the United States." (U. S. P. H. B., 1915).

The disease is caused by the Bacillus Typhosus, which is a non-sporing bacillus, and usually conveyed to man by drinking water, milk, eating food such as vegetables washed with water contaminated by dejecta from typhoid patients as "carriers," (it is stated that 60% of all recoveries are carriers for some time), or food handled by uncleanly carriers, or by the common fly, the Musca domestica. The house fly lays about 120 eggs at a time, in manure, rubbish and filth. The egg takes about 8 hours to hatch into a larva, which in 5 days becomes a pupa and in another 5 days grows into the fly; so that in a total of 10 days from the time the egg is laid, a fly is developed—hence a multitude of flies in a short time. They live one season and the offspring from one female fly in one season may produce 214,844,320,000,000,000,000 flies. Each fly is about one-quarter inch in length; so that by placing these flies in a row, head to tail, the offspring of one fly would encircle the earth's equator over 5,000 times. It takes about 2,000 flies to weigh one pound. Flies can scent food and bad smelling substances from 20 to 30 miles; sweet smells as the odors of flowers are repulsive to flies. The fly does not soar as many insects do, but flies. The muscles are larger and greater in comparison than those of the carrier pigeon. Wherever man is there flies are found. The fly is probably man's worst enemy. The fly has on each foot two claws and two light colored pads—the claws hold to rough surfaces and the pads are covered with a sticky exudation which enable them to hold to smooth surfaces by adhesion. The pads are covered with hair and these hairs are constantly being clogged with minute particles, which must be removed and as they are too sticky to be brushed off, the fly must remove them by licking with its tongue, thus carrying disease. The fly walks over contaminated substances; the hairs are clogged; they are cleansed by the fly; the bacteria and other substances are swallowed; and as the fly defecates about every 30 seconds, the bacteria pass through the digestive tract practically unharmed. At a single meal, a fly will swallow about one-half times its own weight; eating only fluids, it spews a form of gastric juice on the food dissolving the food so that it may pass through the strainer on the end of its proboscis. If the fly

has eaten its fill, but sees or smells something it likes better, it spews out the stomach contents and proceeds to eat the food which has attracted its attention. 95% of flies are bred in filth and in the stables, which could easily be prevented by sprinkling 2 ounces of borax to the garbage can, or 10 ounces to every 8 bushels of manure. If these precautions were observed, we soon would have a flyless country, saving millions of dollars, lives and much suffering. Buy from dealers who screen their food. Fly specks on the windows were examined and found to give about 10,000 colonies of bacterial growth to each speck.

Styles buried house flies in a screened stand pipe 48 inches under sterile sand and they came to the surface through the sand; so that simply covering excreta with sand, ashes or lime, will not prevent breeding of flies.

Typhoid is carried by the oyster which has fed on sewage. The oyster seems to have a preferance for sewage ladened waters, in which it flourishes. It takes about 3 years for the oyster to grow into a marketable stage. A single oyster spawns about 50,000 eggs a year.

Typhoid bacillus is reduced in the rivers in one week 30% in 6 weeks about 99%; and it will live in water for 103 days or more. It is usually killed in milk, when the milk becomes sour, probably being killed by the lactic acid which has developed, or by the lactic and other organisms eating all the food. Typhoid bacillus will live in oysters for 3 weeks, in clothing 3 or more months, and in soil and man indefinitely.

Typhoid carriers are those who never have had the disease, or those who have made complete recoveries, but may harbor the bacillus in the gall bladder for years and yet be apparently healthy, but are eliminating the bacillus in urine or feces. Such persons are called 'carriers'—they are able to infect others. The first carrier was "Typhoid Mary."

In the summer of 1906, there occurred six cases of typhoid fever in the household of a wealthy man at Oyster Bay, N. Y. Diligent investigation of the milk and water supply and of articles of food which might have been implicated were eliminated as possible causes.

The history of the household showed that a new cook had been employed about three weeks before the first case of typhoid appeared. She had left shortly after the outbreak of the fever. She was described as an Irish woman of about forty years, unusually intelligent. She possessed enough skill as a cook to command high wages and had been able to

obtain work in the most desirable situations. She was non communicative, self-reliant and courageous. She was in per fect health apparently at this time. Her name was Mar Mallon. The information collated with respect to the move ments of the woman for the preceding ten years showe that she had worked for eight families, and in seven of thes typhoid fever had occurred. An outbreak in a New Yor family in January, 1907, led to her discovery. She denie having had anything to do with the cases of typhoid whic had occurred in the families where she had been employed and said that, because of her faithful attention to some o the cases, she cured typhoid rather than caused it. Sh would not assist the investigators in doing anything whic would clarify the situation.

Finally the case was laid before the medical officers o the New York City Department of Health, with the sugges tion that the woman be taken into custody and proper ex amination of her excretions be made to determine if she ha been responsible for the many cases of typhoid fever. Thi was done, and despite the fact that no assistance could b gotten from the woman, because she would not talk, the feces were examined. They showed the typhoid bacillus in great numbers. She was virtually a living test tube for the organ ism. She was careless about washing her hands after visit ing the toilet and when she prepared a meal the germs on her hands came in contact with the food.

She was kept practically a prisoner for three years. Two legal actions were brought to secure her release. It was claimed that she was deprived of her liberty without ever having committed a crime or knowingly having done injury to any persons or property; she was apparently under life sentence; people remarked that it was contrary to the con stitution. The case attracted wide attention. The courts agreed with the department of health that they acted within their rights.

Public sentiment was mixed. On one hand Mary was pictured as frying typhoid bacilli as large as sausages in preparation of the family meal. On the other hand she was pictured a lonely prisoner with only a cur for a companion. There was much sentimental sympathy, the kind bred by too much democracy or near bolshevism. In 1910, when a change occurred in the administrative department of the board of health, Mary was released, with the understanding that she would not engage in cooking or in any employment which necessitated her handling food supplies.

For a while Mary kept her promise, but finally broke her parole and disappeared. She was lost sight of for five years, coming to light again in 1915 when an outbreak of typhoid occurred in the Sloane Hospital for Women in New York City. In this epidemic there were twenty-five cases, mostly among the nurses and attendants. Mary was found to be the cook. She was known in the hospital as Mrs. Brown, but when the epidemic occurred was jokingly nicknamed "Typhoid Mary" by employees who remembered the published accounts of Mary Mallon. It was this nickname which led to her apprehension. When she became aware of the fact that suspicion was directed against her, she again disappeared, moving to New Jersey and then to Long Island. She was finally arrested and forcibly brought into custody at the Riverside Hospital, where she is still under detention.

Mary Mallon caused ten known epidemics of typhoid and a total fifty-one known cases of the disease. These represent only a part of the total damage she did to society.

Her case is of interest because it brings to light the ways in which disease may be spread without the real cause being suspected.

In the Spanish-American War fully 65 per cent of all cases of typhoid were hand-borne; that is, they were contact cases from man to man. In the present war as much attention was devoted to the isolation of "carriers" as to sanitation and inoculation. This case proves, too, that our food is frequently contaminated by the excrement of some person or persons. It demonstrates how much attention should be paid to the washing of the hands after leaving the toilet and always before eating or handling food. It also protests against the habit of handshaking.

The case of "Typhoid Mary" is an argument in favor of typhoid inoculation, for we do not know when we may, in our travels, cross the path of a typhoid carrier.—From the August, 1919, Till and Tile.

Then followed Typhoid Rosie, in Chicago, who had recovered from the disease but was still voiding the bacillus, and not being very clean in her habits, polluted food and in this way infected a number of people.

The great epidemic in Strassburg was caused by six women, who had the disease from 1 to 27 years before. The typhoid bacillus has found in the sputum of 78.5% of the recovered cases; and in the feces of 21% cases; and in the urine of 94%; hence uncleanly cooks are especially dangerous.

The bacillus is harbored in the gall bladder, and is one

of the causes of hobnailed liver. It may cause chronic diarrhoea by being poured through the gall duct to the intestines; and is said to be the cause of gall stones. On examining a gall stone microscopically, a small cavity is found resembling the shape of the typhoid bacillus. In many cases typhoid inoculations produce symptoms of gall stones.

A law has been passed in nearly all of the states compelling the people who handle food to be examined at stated periods. Safety first—wash your hands before eating or handling food.

About 1884 typhoid fever and typhus fever were separated into two distinct diseases. Typhoid fever is infectious but not contagious, and the symptoms of the disease are produced by a toxin,—typho toxin, and it is a disease of the lower third of the intestines; hence the name Enteric fever.

TYPHUS FEVER. Brill's, Tarbadill's, Ship, Camp, etc., fever. This is a disease with many names,—the organism causing this disease was thought by Rilapo in 1828 and by Obermeier in 1836 to be a spirocheta, but now known not to be the cause. In 1909 Nicolle infected animals with the disease by injecting blood from patients with the disease. Experimentally monkeys can be infected and after recovery have an immunity lasting about two years. The organism causing this disease is carried by the louse. The incubation period of the disease is 4 to 5 days; and as the name typhus signifies a cloudy mind, is one of the first symptoms, accompanied by headaches, chills and high temperatures, and the whole body is covered with the "mulberry" rash in the 4th day, and about the 7th day high delirium, sometimes followed by unconsciousness, contracted pupils, generally weakened condition sets in with the crisis about the 14th day, if the patient lives this long. The temperature falling, reason returns rapidly; few relapses are known. The death rate is from 25 to 50%. Treatment is good nursing. Prophylaxis consists in not overcrowding camps, ships, etc., and keeping these places in a sanitary condition, and free from vermin.

Typhus fever has appeared in scattered cases in nearly every country each year for many years. In the last several years Russia has led in the number of cases; then Galicia; then Poland; and the disease has been epidemic in the Balkans and Turkey in Asia. Typhus cases appear every year in Persia, northern China, Siberia and Japan. During 1769 to 1833 in England there were over 23,000 deaths from typhus fever; and from 1884 to 1898 there were 2,249 deaths, and

from 1898 to 1913, 390 deaths. In France from 1903 to 1913 there were 209 deaths. The disease appears less frequently in the Scandinavian countries. In the recent epidemic in Siberia, it is estimated that 135,000 persons died from typhus fever; and in Russia from 1905 to 1911, there averaged each year from 4 to 11,000 deaths. It is found in Northern Africa, but rarely as an epidemic. Egypt has annually about 1,500 deaths; and in the epidemic of the first 6 months of 1915 there were 15,000 cases and 4,000 deaths. The louse as being the carrier of the organism which causes the disease was discovered at Tunis, and later confirmed by our investigators in Mexico, where there were from 1893 to 1913, 57,000 cases and 15,000 deaths.

The Weil-Felix reaction for typhus. The reaction consists of agglutination with the "proteus-like" organism. Although an extraordinarily interesting reaction and of very definite value for purposes of diagnosis, the Weil-Felix reaction will still have to be classed, with the Widal, as an aid to diagnosis only. From the fact that a positive result in a diln. of 1:50 is given by 8% of normal individuals, 25% of patients with fever other than typhus and typhoid and in nearly every case of typhoid fever, it is obviously unsafe to make a diagnosis unless a higher titer than this is obtained.

The Typhoid Bacillus, or Bacillus Typhosus, is an easily cultivated Gram negative non-sporing, motile bacillus, appearing as a straight rod, with rounded ends and from 1 to 3 by 0.5 to 0.8 microms. It was discovered in 1880 by Eberth and grown by Gaffky in 1884. It grows on ordinary media, producing no indol, or gas but acid in dextrose broth. In agar it grows on the surface as thin blue-white irregular notched margined colonies.

The typhoid bacillus is destroyed in river water by the saprophytes and sunlight. It enters the body usually by the gastro-intestinal route, and effects chiefly the lower third of the intestines, and after an incubation of 14 to 30 days the symptoms appear. The bacillus may be obtained from the blood until about the 15th day. No leukocytosis is found (different from appendicitis). Polymorphonuclear leukocytosis is found in intestinal perforation only. An immunity is produced, which lasts for some time, even after all agglutinating power has been lost by the blood. An immunity is conferred by the mother to the babe. The lymph channels are invaded through the blood.

Hexamethylenamine causes the typhoid bacillus to disappear from the urine.

Typhoid fever rarely occurs in adult life; and it has an incubation period of 14 to 30 days, with symptoms of weakness, headache, diarrhoea (none in typhus fever) a high fever, (low in the mornings), at first vomitings, back and knee aches, the rose rash on the abdomen in the second week, which lasts usually 4 days, and the duration of the disease is about 4 weeks. Convalescence is slow (typhus rapid), perforations of the intestines may occur, also delirium in many cases. There are few cases in the tropics, but more in the semi-tropics. Every case is always caused by infection from a previous case; and every case can be directly traced to another case. It is a disease of carelessness or dirty habits.

In typhoid fever a secondary anemia appears with a loss of 5 to 6% hemaglobin per week for the first 5 weeks. Leukopenia occurs especially in the second week. The urine undergoes rapid decomposition and becomes turbid on standing at room temperatures, and urate deposits develop. The reaction at the first stage of the disease is acid and on convalescence it is alkaline. A small number of hyaline and finely granular casts and pus cells are found. In some cases blood is found in the urine. Typhoid bacillus is found in the urine and for sometime after recovery.

Prophylaxis. Typhoid fever should be stamped out by personal cleanliness and care in disposing of dejecta. Anti-typhoid serum has been used with but little success. Injections of typhoid bacterial vaccines have been used with great success, and an immunity is produced which lasts from 2 months to 2 or more years. Wright first used the bacterin in 3 doses, one billion killed typhoid bacilli, followed in 7 days with a dose of 2 or more billions; and in 7 days with a third dose of one billion. It was tried in the German army with great success; then in the English army, and now is used in our army with excellent results as a prophylactic. We use killed cultures of typhoid and para typhoid bacilli, with ½% tricresol added as a preservative.

Deaths from typhoid fever and the results of vaccination.

In the Franco-Prussian war 60% of the deaths were caused by typhoid fever.

In the Boer war, 5,877 British soldiers died out of 31,000 cases. There were 8,022 deaths from all causes, and 7,781 deaths among the Boers.

The British army in India in 1911, consisted of 61,622 men. 1.7% had typhoid fever. Of the immunized only a fraction of one per cent contracted fever and these had only

mild cases, and the death rate was 0.17% per 1,000; while the death rate of non-immunized was 1.15% per 1.000.

In our Civil War the Union had 93,369 killed and 186,216 died of disease.

In the Crimean war 4,602 were killed and 17,580 died of disease.

In the French Madagascar expedition 29 were killed and over 7,000 died of disease.

In the Spanish-American war 545 were killed and 5,277 died of disease.

In the Japanese army in 1909, there was one case of typhoid fever per 1,000 in 12,915 immunized men; and 14.52 cases per 1,000 in 20,245 not immunized men. A reduction of 93% among the immunized.

The German army in 1913 had 21 cases and one death out of 5,473 men immunized and 176 cases and 26 deaths out of 6,610 not immunized men.

In the United States army during the Spanish-American war there were 107,973 men and 20,738 cases of typhoid fever with 1,580 deaths, or nearly one case to every five men. But in 1911 during the four months at San Antonio, Texas, 12,800 men were immunized and only one case resulted in death, and this one case had not completed the immunization. During the time the soldiers were there, 19 civilians of the city died of typhoid fever. In the first 60,000 U. S. soldiers immunized, there were only 12 cases and one death.

In Florida in 1898 there were 2,000 cases among 10,000 soldiers; and in 1911 there were 20,000 soldiers immunized, and only two cases and one death.

In 1912 the U. S. had 58,119 soldiers; all were immunized and only 12 cases. The death rate was 3 in 100,000; while during the same period, the civilian death rate was 22 per 100,000.

The United States had over 35,000 cases in registered areas in 1918, or one person in every 200 had typhoid fever.

In 1910 there were 565 cases per thousand.

In 1914 there were 0.003 cases per thousand; and in the army 88,178 soldiers, home and abroad, all immunized. There were only eight cases and no deaths.

In 1915 there were over 100,000 persons immunized; a publicity campaign, world wide, was started and if this had continued by 1916, typhoid fever would have been a disease of the past.

In 1913, by immunization in the army the mortality was a very small fraction of 1%, while in 1909 the mortality was

30.3%, and in 1912 it was 0.3% with less than 5% recurrences per thousand.

In 1912 the civilian population had over 400,000 cases and 30,000 deaths.

In 1913 in the registered world area there were over 150,000 deaths; and in the United States 25,000 deaths, or 10% of the cases died, causing a loss of over three million dollars from a preventable disease.

In 1916 our soldiers in Texas had only three cases out of 30,000 soldiers.

Up to October 19, 1917, out of the million men in camps, all were vaccinated, and there were 12 cases developed. While the British army had only 0.1 cases per thousand.

In the Spanish war there were 20,000 soldiers landed at Porto Rico, which had a population of 17,000; and there were three soldiers killed and 262 died of disease; or nearly 100 times as many died of disease as were killed.

In 1898 our army at home and in Cuba and Porto Rico had 293 killed, and 3,681 died of disease,—4,422 cases of typhoid and 248 deaths.

There were 170,000 men enlisted for the Spanish war; of this number there were 158,000 hospital admissions, or 90%—three fourths of these men never left the United States during the three months war. Poor food and unsanitary conditions are given as the cause of this large hospital admission.

Japan army during this same time had 4% hospital admissions. (Rept. Surg. General Oct. 1905).

In our last war, all our soldiers were inoculated and typhoid fever was practically eliminated.

During 1915, 8,124 patients in hospitals in California were vaccinated and the death rate was reduced to 0.49 per thousand.

Vaccination will not absolutely protect in all cases against typhoid fever, but it will reduce the susceptability, the duration and severity of the disease, reducing mortality, and is absolutely harmless if properly used, even if no beneficial results are obtained. The earlier the vaccination occurs the better it is; the dose must not be too small, as small doses tend to cause anaphylaxis. In cases of fever it will reduce somewhat the duration, and lower the temperature.

It is estimated that over 19,000 lives have been saved in Pennsylvania by this vaccination in 1914.

The excreta-stools and urine from patients must be sterilized with steam, or if this is not possible, a cup full of un-

slacked lime must be added and a cup of hot water poured over the lime, the vessel covered and left stand for 1 hour; or formaldehyde may be added and left stand for 2 hours; or phenol 5%, formaldehyde 10% and lime 6% are very good. The rooms and furniture washed and directed under fumigation of rooms.

TESTS FOR TYPHOID FEVER

1. The atropine sulphate test. One hour after eating, the patient lies horizontally, relaxes and rests until the pulse has become even. The pulse is counted minute by minute for 10 minutes; then a hypodermic injection of atropine sulphate about 2 mgm. (1-30 grain) is given. In 25 minutes the pulse is counted as before. Example—if the pulse was normal at 70, and after injection was 78 the result is positive; or an increase of 15 beats or over per minute indicates a positive; but if a rise of less than 15 beats per minute the result is negative.

2. Agglutination tests, which may be Macroscopic or microscopic (See pages on).

3. Opthalmic test. A 24 hours' culture of typhoid bacillus is centrifuged, wash in salt solution; killed at 60 degrees C.; ground with NaCl crystals; macerated 3 days in alcohol and 1 gram of the residue is mixed with 1 mil of alcohol. One drop of this is placed in the lower eyelid; if in 6 to 8 hours inflammation occurs, the reaction is positive.

4. The skin test. Five million or less killed Bacillus Typhosus injected under the skin, and if in 2 to 4 hours redness appears, a positive reaction is indicated; or if redness is delayed for 12 hours and disappears in 48 hours. Redness lasting more than 48 hours indicates an infection. Great care must be taken in making the injection so that no blebs are raised.

5. The Typhoid Quotient. If the spot in test No. 4 after 24 hours is 1½ times as large as the control spot, the quotient is said to be 1.50; when above this, immunity to typhoid is suggested; while if below this number there is a non-immunity.

6. Epidermal test. The scarrified skin is rubbed with a little of the killed bacterial emulsion; if redness results a positive reaction is indicated.

The above tests with the exception of the microscopical agglutination test are not reliable and some may be harmful.

7. The Urichromogen or Russo Urine Test: To 5 mils

of urine add 4 drops of an equeous solution of methylene blue; an emerald green color indicates a positive reaction and a blue color a negative reaction.

8. The Weiss-Rhein Urine Reaction consists of mixing equal parts of urine and water then add 3 to 10 drops of a 1 in 1,000 potassium permanganate solution, (or a small crystal may be used). If the solution turns brown, it is a negative test; but if a golden yellow, the test is positive. It is best to always use controls.

The Precipitin Test. Germ free filtrates from broth cultures are mixed with their respective antiserum obtained from immunized animals, give precipitations. The germ free filtrates must be from the same bacteria as that from which the animals have been immunized, to obtain the serum; as typhoid filtrates will not precipitate cholera serum. This substance in the immune serum which causes the precipitation is called precipitinins. Some albuminous bodies when injected into animals form the corresponding antibodies, which have the power of precipitating the substance used for inoculation; as cow's milk when injected forms a precipitin which will precipitate cassein from cow's milk but not cassein from other milks. Serums of animals injected with human blood, when mixed with human blood even in high dilutions will cause a precipitation, but only with human blood—this is a reliable test for blood of various animals, and used in murder cases. Other animal serums cause precipitates with the same animal blood.

THE AGGLUTINATION TEST

The blood of a typhoid patient when mixed with a culture of the typhoid bacillus will cause a clumping or agglutination of the bacillus, in groups of 4 to 30.

The discovery of Widal and Gruber that this agglutination appeared in typhoid serum, even in very high dilutions gives us a ready means of diagnosis. The agglutination is usually positive; but in some cases may be absent. Positive material is always obtained from the spleen; and the agglutinating bodies are found in blister serum, the patient's milk, blood and fetus serums.

1. The Macroscopic Test—Bed side test. Consists of depositing in each of a series of small test tubes, 1 mil of salt solution. To another test tube add 1 drop of the patient's serum and 4 drops of salt solution. This forms a dilution of 1 in 4. This is well shaken and 1 drop of this solution is added to another test tube with the same amount of salt

solution. This makes a dilution of 1 in 16. This method of dilution is continued until a dilution of 1 in 128 is secured. To each of the tubes in the series with salt solution, add 1 mil of an 18 hour old broth culture of typhoid bacilli (or dead organisms may be used but they are not so good),—add the different dilutions of the serum, labeling carefully each tube. A precipitation visible to the eye may occur in some cases in the lowest dilutions within several minutes, but it is best to place the tubes with the dilutions in the incubator at 37 degrees C. for 2 to 8 hours before reporting negative. If the reaction is positive, agglutination occurs, causing the bacilli to clump together and gravitate to the bottom of the test tube; the upper liquid remains clear. If turbidity results, the test is negative. Control tubes must always be carried. When agglutination occurs, dilutions of not less than 200 are to be considered positive.

2. MICROSCOPICAL TEST. This is delicate and reliable.

Use only 16 hour old broth cultures; if dead organisms must be used, they are prepared by adding normal salt solution 450 parts, phenol 2 parts, glycerin 50 parts to the broth culture; incubating 2 hours and shaking to break up the clumps.

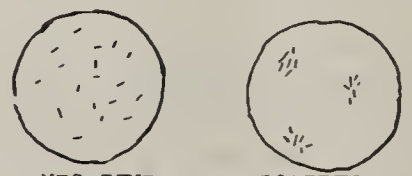
Use great care and follow the outline word for word.

1. Clean a dish slide and cover glass.
2. Ring slide with vaseline.
3. Flame platinum needle and add a loopful of the culture to the CENTER of the cover glass—a small drop only.
4. Flame needle and add a loopful of the blood to the culture on the center of the cover glass; mix carefully. Flame needle.

I.CAPILLARY TUBE. 2.BLOOD FILLING TUBE. 3.FILLED TUBE
3. ENDS SEALED IN FLAME.
OBTAINING BLOOD FOR THE WIDAL REACTION.

5. Invert slide over the cover glass, use great care.

6. Examine with low power to locate the hanging drop; then use high power; you will see the bacilli shooting across the field; but soon the reaction takes place and the bacilli becomes sluggish or fixed in one place. The slide should be placed in the incubator for 30 minutes and then examined for the clumps. When clumping or agglutination occurs in blood diluted 1 in 50, the reaction is 1-50 positive. But if no clumping occur, the reaction is negative. In actual practice dilutions of the blood are made on a cover glass, ruled like the spokes of a wheel with a grease pencil, so that dilutions are obtained from 1 in 50, to 1 in 250. The dilutions are made with normal salt solution or sterile broth. Usually when the reaction is obtained in dilutions of 1 in 30, the reaction is given as positive; but it should occur in dilutions of 1 in 250 to be absolutely positive.



NEGATIVE POSITIVE

GRUBER-WIDAL AGGLUTINATION REACTION.

7. After performing this test, carefully remove the cover glass as directed under the hanging drop exercise,—fix in the flame and stain by the Gram stain. Great care must be used. Go slow, understand each step before performing it. If bacteria touch slide or anything else, notify the instructor at once.

The Widal reaction is obtained after immunization in 1 in 50 dilutions and from 1 to 6 months after vaccination a strong positive reaction is obtained; after 12 months about 50% of the reactions are positive; after 36 months about 40% are positive; after 48 months 30% are positive and after 50 months only 1% is positive.

A positive reaction in patients who at some time have had typhoid fever or inoculations is of little value in diagnosing typhoid fever.

Blood cultures and total and differentia blood counts are of value.

The University of Kansas tested the agglutinating power of their students' blood with these results: Before inoculation all gave a 4 to 6 dilution positive, and two weeks

after vaccination all gave from 64 to 128 dilutions; after one year all gave positive reactions of from 32 to 128. The immunity thus conferred produced reactions for three years. So when we perform the reaction, to make the diagnosis correct we should know whether the patient had not had the disease or inoculations for at least three years previous to the time of making the test.

Sensitized Vaccine. Agar cultures of a number of strains of typhoid bacilli, washed with 2.5 mils of sterile salt solution for each slant growth; the washings containing the bacteria are mixed and sealed in a sterile tube.

The bacteria are grown in special culture flasks which afford a large surface for growth.

Submerged in water at 60 degrees C. for one hour to kill the bacteria and tests for sterility made. The number of bacteria to the mil are estimated and serum from the blood of animals such as goats, which have been immunized to the disease by the use of the same strains of bacteria, is inactivated at 56 degrees C. for 30 minutes; then 2 mils of this serum is added to 1 mil of the bacterial emulsion, shaken, covered with toluol, incubated 24 hours at 37 degrees C., with frequent shakings. It is then gently shaken and centrifuged for 5 minutes, washed with salt sodium and centrifuged a number of times, and is diluted so that each mil contains 500 million sensitized bacilli. Inoculations are made about every 3, 5 and 7 days, in doses of 2 to 500 million. It is said to have been used with good results during the disease and convalescence.

CULTURING THE BACILLUS TYPHOSUS

1. From the patient's blood. Tubes containing 10 mils of sterile ox bile are inoculated with 2 to 5 mils of blood. They are incubated 48 hours, when they are shaken, and Endo-agar paltes are inoculated, and these are incubated for 24 hours, when the suspicious colonies are subcultured on Endo's medium for 24 hours.

Or blood is taken in a sterile syringe from the cubital vein and placed in tubes containing ox bile 9 parts, glycerin 1 part, and peptone 2 parts.

Or the blood may be plated on agar medium containing 1% sodium glycochocolate.

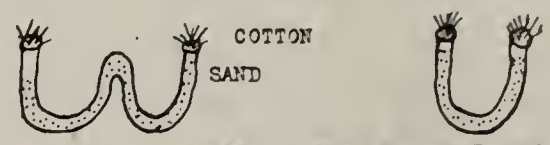
2. From the patient's urine. Several drops of the urine are inoculated on Endo's medium and suspicious colonies are subcultured twice; then they are subcultured in glucose broth with litmus to determine gas production; if acid is produced,

ground, while coli appear as red colonies on and below the surface, and produce acid and bubbles of gas; the paratyphoid produce acid and gas on the bottom and are blue on the surface; dysentery bacilli appear similar to the typhoid.

Brilliant green medium. Add to agar medium which has been neutralized to 0.7% acid, 1% lactose, 0.1% glycerin and sufficient 0.1% aqueous solution of brilliant green to color.

Typhoid bacillus growing on brilliant green after 18 hours incubation appears by reflected light as grayish colonies; coli appear as red colonies. By transmitted light typhoid colonies are transparent and colorless; coli have very dark centers and are reddish. By further incubation the typhoid colonies become pink and transparent, while the coli become dark red, or purple and even more opaque.

Sand Tube Separation of Bacillus Typhosus. This test depends on motility. A sterile sand filter through which the bacilli penetrate and pass through sand (filtered through a No. 40 sieve) in 18 to 24 hours. The typhoid bacillus passes through this tube filled with sterilized sand and broth, which has been tinted red within eutral red, and if present may be obtained in pure cultures from the opposite end and cloudy from that which had been inoculated.



TUBE 33 cm.x 6 mm. FILLED WITH NUTRIENT BEFF BROTH.
FOR THE ISOLATION OF BACILLUS TYPHOSIS.

Gentian violet medium. To litmus lactose agar medium add a 1 in 100,000 aqueous solution of gentian violet. Alkali-producing bacteria are inhibited.

Holt-Harris medium. Litmus saccharose agar medium is treated with dilute solutions of methylene blue and eosin. After 18 hour incubations typhoid colonies are colorless and transparent; coli are deep colored, almost black and do not transmit light, and the surrounding medium is unchanged.

Toyoda's dessicated ox-gall medium. For blood cultures use pulverized ox-gall 3 grams, peptone 1 gram, sodium chloride 0.5 gram and water 100 mils. For cultures from samples of water,—to every 100 mills of the water add peptone 1 gram, sodium sulphate 2.5 grams, sodium chloride 0.5 gram mixed with 7 mils of distilled water. This is incubated at 37 degrees C. for 8 hours to give the typhoid bacilli time

to multiply; it is then treated with a solution of dessicated gall 3 grams (dissolved in 7 mils of distilled water) and 1 mil of a solution of 0.1 gram of crystal violet in 8 mils of distilled water. This checks the development of nearly all other bacteria, so that after standing 10 to 16 hours at 37 degrees C., if a small amount is transferred to Endo plates test the culture with agglutinating serum, of typhoid and paratyphoid A and B.

3. From the patient's feces. Specimens are collected on diphtheria swabs and inoculated into broth; or pieces of the stool the size of a pea may be rubbed in 10 mils salt solution, let stand 15 minutes; then a number of loopfuls from the top are inoculated on the surface of an Endo agar plate —Incubated and examined for the typical small colorless colonies.

Endo's medium is made by making the nutrient agar 0.2% acid to phenolpthalen and adding 1.8 mils saturated aqueous solution of fuchsin to each liter, cooling and adding 10 grams of lactose. The medium should have a faint purple tint. Then add a 10% magnesium sulphate solution until the medium assumes a pale rose color when warm. (About 25 minims to the L.)

Russel's Fermentation Test or Russel's Double Sugar Medium. A 5% solution of aqueous litmus is added to nutrientag or until it is purple colored; then add NaOH solution until it is neutral to litmus; then 1% lactose and 0.1% glucose are added. The medium is tubed and sterilized for 15 minutes a day for 3 days. A streak or stab culture of typhoid bacilli appears as colorless coloneis on a blue the typhoid bacillus is easily isolated. To make the dessicated gall, fresh gall is heated in the Arnold, filtered, dried and pulverized.

Kendall and Ryan's double sugar medium contains 1% of saccharose and 0.1% mannitol. It distinguishes between the various saccharose acid and gas and the mannitol acid and gas producers.

Paratyphoid is a typhoid fever-like disease of which two varieties are recognized, the alpha (A) and beta (B). The type B is more widely distributed.

The paratyphoid bacillus is a small rod-shaped organism, with rounded cells and peritrichae flagellated; it is non-sporing and much more motile than the typhoid bacillus. It will not liquefy gelatin, and will cloud broth uniformly.

It grows on solidmed ia aspale moist translucent, bluish-
cast colonies and on Endo's medium ppears very similar

nus milk in 4 days,
ce acid in 14 days,
he acidity turns to
ose or dulcite, while
lead acetate agar
slightly pathogenic

SOME SUGARS FERMENTED BY THE COLON-TYPHOID GROUP OF GRAM-NEGATIVE-NON-SPORING BACILLI.

	Bacillus	Hexose	Mannite	Maltose	Arabinose	Rhamnose	Sorbite	Xylose	Dulcite	Lactose	Saccharose	Raffinose	Salicin	Inocite	Dextrose
GROUP No.1 will not ferment carbohydrates higher than hexoses.	alicaligenes	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	Shingae	+	−	−	−	−	−	−	−	−	−	−	−	−	−
	Morgani	⊕	−	−	−	−	−	−	−	−	−	−	−	−	−
GROUP No.2 will ferment hexoses, mannite and sometimes other carbohydrates but not lactose. No gas is produced.	dysenteriae	+	+	±	±	∓	±	∓	∓	−	∓	∓	∓	±	±
	var. Flexner	+	+	+											+
	var. Hiss-Russel	+	+	−							−				+
	var. Strong	+	+	+							+				−
	typhosus	+	+	+	−	−	+	+	−	−	−	−	−	−	+
GROUP No.3 will ferment hexoses, mannite, arabinose and rhamnose but not xylose or lactose. Gas may be produced.	para typhosus A	⊕	⊕	⊕	⊕	⊕	⊕	−	⊕	−	−	−	−	−	−
	Pullorum	⊕	⊕	−	⊕	⊕	⊕	−	−	−	−	−	−	−	−
	variety	+	+	−	+	+	+	−	−	−	−	−	−	−	−
GROUP No.4 will ferment hexoses, maltoses, mannite, rhamnose and xylose but not lactose. Usually no gas is produced.	Gallinarum	+	+	+	+	+	+	+	−	−	−	−	−	−	+
	Pfaffi	+	+	+	+	+	+	+	−	−	−	−	+	−	−
	Jeffersoni	+	+	+	+	+	+	+	−	−	−	−	−	−	+
	aertryke	⊕	⊕	⊕	−	⊕	⊕	⊕	⊕	−	−	−	−	−	−
	enteriditis	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	−	−	−	−	−	−
	para typhosus B	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	−	−	−	−	⊕	−
GROUP No.5 will ferment hexoses, mannite, pentose, and lactose, producing acid reactions and a small amount of gas.	acidi-lactici	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	−	−	−	−	−	−
	var. Grunthal	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	−	−	−	−	−	−
	coli communis	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	−	−	−	⊕	−	−
	var. immobilis	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	−	−	−	⊕	−	−
	communior	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	−	−	−	−
	var. coscoroba	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	−	−	−	−
	neapolotanus	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	−	−	−
GROUP No.6 will usually ferment all carbohydrates except dulcite and inocite which vary. A weak acid reaction is produced and much gas	cloacae	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕
	aerogenes	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕
GROUP No.7. Organisms producing reactions similar to group No.6.	proteus	⊕	−	⊕						−	⊕				−
	Friedlander	+	+	+	+					+	∓	+	+		+
GROUP No.8	para typhosus C														

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Positive + Usually positive ± Gas ○
Negative − Usually negative ∓

	GELATIN
	−
	−
	−
	−
	+
	+
	+
	+
	+
	+

n water which had
sterile bottles, and
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ius Penicillium. B.
during this period
d under favorable

water supply has
, Quincy, Ill., 1913
Salem, Ohio, No-
pulation contracted

RABIES—(HYDROPHOBIA)

A specific disease to which man and all animals are susceptible and communicated by direct infection. Man is infected by the bites of dogs, cats, squirrels, etc., but may contract the disease from handling diseased animals. The virus is present in the saliva of the infected animal and is demonstrable from 24 to 48 hours before symptoms appear. The disease is caused by a filterable virus, from which Dr. Leteve isolated the organism in 1907 and in his honor was called Plasmodium Leteve.

It is a disease of the nervous system and bites causing the infection, upon unprotected parts of the body having rich nerve supply, are more serious than those on the parts protected by hair and clothing and having less nerve supply. The virus is found in all the nervous system and the medulla of the brain. It is destroyed by heat, and when dry, in 48 hours by sunlight.

The incubation period may vary from a month to a year. The symptoms are those of primary depression followed by great excitability when excessive sensitiveness to external stimulants. The oral and laryngeal muscles become subject to painful spasms excited by the least irritation, such as attempting to drink,—this inability to drink on account of the contraction of the throat muscles gave rise to the term Hydro—water and fobia—fear, which is a misnomer. Loud talking, shutting of a door, etc., causes spasms. Hysterical rabies or lyssaphobia symptoms closely resemble those given above but they are exaggerated and ill-timed as the first symptom may be that of a dog bark, the patient going about on all fours and biting at the furniture. This is not caused by a virus or micro-organism but exists in the patient's brain and may be cured by suggestion. A good test is to step up quickly behind the patient and fan him with a fan so that the air strikes the back of his neck; if this brings on a convulsive seizure it is an indication of true rabies.

Since the days when the Greeks worshipped the stars, the disease has been known. Hydrophobia means fear of water and is a misnomer, so called on account of the suffering animal having constrictions of the throat or spasms of the throat, and being unable to swallow, and not due to its fear of water as the ignorant believe.

To appease the wrath of the gods, the Greeks on first beholding the star Sirus, sacrificed a brown dog—hence the term dog days. At this season of the year the days become

hot and sultry; and the people said "wine grew sour, dogs became mad and man became afflicted with fevers, hysterics, frenzies and all manner of things." At Argos a festival was held called Cynophantes—meaning that all dogs were to be killed at sight. But we now know that more dogs become rabid in cold weather as in winter than in summer. Dogs have fits in the summer and usually cold water thrown over the dog will restore him. The Italians used the hair of the dog which had bitten the patient to cover the bite; and they were not so far from the treatment, for at one time the vaccine was made from the cord of the rabid dog and injected into the infected person. Later came the period of mad stones, mad pills, et al. These were of no value in the cure of the disease; for there has been no cure discovered; the disease is always fatal after the manifestations appear; but thanks to the father of bacteriology, Pasteur, a preventative is known. In 1884 Pasteur achieved his great accomplishment, vaccinating a boy, Joseph Meister, July, 1885. In 1888 the Pasteur Institute of Paris was organized; and today there are over 60 recognized institutions.

If the wound caused by the bite of a rabid animal is opened and cauterized with nitric acid the danger is lessened; as there were 89 deaths out of 249 cauterized wounds, to 96 deaths out of 117 uncauterized wounds. A record of 24 Pasteur institute shows that 54,620 cases were treated, with a death rate of 0.77,—less than one per cent. Bernstein reports a record of 104,347 treatments with a death rate of 0.54 %. Dr. Leteve. one of the world's greatest bacteriologists, a student of Pasteur in Lille, France, and Director of the Pasteur Institute in Pittsburgh, in 17 years' treatment, had a death rate of less than one-tenth of 1%. In 1907 Dr. Leteve after 20 years of investigation discovered the cause of rabies, which is a protozoon and is called Protozoon Leteve. Previous to this time no specific cause was known, except the cause was a filterable virus. In 1903, Negri bodies, which are certain changes in the brain and cord of infected animals were discovered by Negri, who claimed these bodies were protozoon parasites; and later Dr. Leteve proved that these were the results of the infection and not the infecting organism. Negri bodies appear as a complete circule of chromatoid granules about a central body and are always and only found in rabies. These bodies are easily seen in specimens by placing a small piece of the gray matter of the brain—Ammon's horn, the cerebellium, or the cerebral cortex on a slide and spreading evenly with another slide.

It is fixed without being permitted to dry in methyl alcohol (neutralized with sodium carbonate 0.25 grams to 500 mils of alcohol) to which 0.1% picric acid has been added, for 10 seconds, the excess blotted and stained with the solution composed of saturated alcoholic solution of fuchsin 0.3 mils, saturated alcoholic solution of methylene blue 2 mils and distilled water 30 mils. After adding the stain to the slide, the slide is held over the flame until it steams; then it is washed with water and blotted. Negri bodies are stained magenta, the nerve cells are blue, and the red blood cells yellow or salmon. This stain must be made fresh as used, or kept on ice. If Negri bodies are not found, the cord may be rubbed in salt solution and about 0.25 mils is injected into rabbits; such emulsion remains active for months when kept in the ice box.

The Pasteur treatment is based on the fact that the rabic virus in the spinal cord of the rabbits loses strength at an even rate of ageing. A fixed virus is one that is so virulent by successive passages through rabbits that it will produce death in about six days; there is no increases in virulence; hence the name fixed virus. Street virus is attenuated for man, but is more virulent for animals. These are filterable viruses. The spinal cord of rabbits which have been killed by inoculations of fixed virus into their brains, is removed aseptically, cut into pieces from 1 to 8 cm. long and dried over caustic potash, in the dark, for 14 days at 23 degrees C. Emulsions are made by rubbing a piece of the cord in salt water and injected every day into alternate sides of the anterior abdominal wall, for 21 to 30 days, depending upon the form of rabies. Beginning with the attenuated cord—that is the 14 day cord 2.5 mils of the emulsion are injected, gradually increasing until the 7 day cord is used. The cord will keep in glycerin and sterile salt solution for some time if kept in the dark and cool. This will produce an active immunity. And this preventative treatment must be given at the earliest possible time after infection. In severe cases the treatment may be given 3 times a day for 4 days, then once daily.

Vaccines are prepared and sent by mail; but as yet, this method of treatment is not as satisfactory as the personal treatment.

Pasteur treatment has been modified in many ways, some of which are: Babe's method of attenuating the virus by heat; Calmette's, the virus attenuated by glycerin; Fermi's, the virus attenuated by phenol; Hogyes' diluted fresh

virus; Ferran's used the unmodified vaccine in large doses Roumanian used a serum vaccine; Marie mixed the viru and serum, injecting daily for three days;—this is the simul taneous method. Harris used the brain and cord of rabbit dying from the fixed virus, grinds to a paste, freezes and pul verizes and dries the frozen mass in vacuo; seals in glas tubes in vacuo; and kept in a cold place, and the standar is the M. L. D. for a full grown rabbit, or guinea pig; thi M. L. D. is the unit. 250 units are used as the first injection gradually increased to the 7th day, and after that 2,000 unit are given.

The Pasteur treatment is harmless, but the virus ma be dangerous if introduced into wounds.

Muzzling of dogs prevents many cases and the numbe of cases decrease when the muzzling laws are enforced. Th Scientific American states: "New York City has a dog popu lation of over 500,000, and less than 100,000 are licensed In 1915 there were over 3,500 persons bitten by dogs." I England from 1887 to 1896 there were a number of rabi deaths yearly; but in 1901, a rigid law was passed, compel ling all dogs imported into that country to be licensed They must be quarantined for six months by the govern ment, and all stray dogs killed; as a result there have bee very few cases of rabies since 1903. until the outbreak i April, 1919, for which the soldiers bringing dogs into th country are blamed.

Brazil in 1911 had over 4,000 cattle and 1,000 horse die of rabies from infections by rabid bat bites.

The rabid dog travels long distances. One dog wa traced from Blairsville, Indiana County, to Harrisville, But ler County, then through the country with many side trip to near Beaver Falls, Beaver County, where he was shot This dog bit many animals along the way; undoubtedl some of these became rabid, and thus the cycle is con tinued.

All animals suspected of rabies should be tied up fo some time, and carefully watched. In case of death, th head should be sent to the nearest Pasteur Institute. I case a report of rabies is received, the bitten person shoul take the treatment at once. This treatment is harmles and is taken yearly by the laboratory workers as a prophy laxis. The treatment is a preventative only, not curative If a patient shows the symptoms, nothing is known tha can save him; but he will die one of the most horribl deaths of convulsions. Care must be taken in handling pa

tients; for in the convulsions, which may be exceedingly great, the jaws may snap and the patient may bite the hands of the nurse. The saliva from the patient is especially dangerous. Bodies of infected animals should be buried at least 15 feet under the ground and the bodies covered with lime, for the earth worms may bring the protozoa to the surface and infect man or animals.

VENEREAL DISEASES

The two great venereal diseases are Gonorrhoea and Syphilis. These diseases cause over one-eighth of all human disease and suffering, causing the deaths of 250,000 persons and 500,000 prostitutes. 60% of the inmates of insane asylums have one or both of these diseases; 50% of all males are, or have been, infected at some time in their lives; 60% of the sterile marriages are caused by these diseases; also 80% of children born with unseeing eyes, or becoming blind a few days after birth; 25% of the inmates of blind asylums; 80 out of every 100 women who die of diseases of the reproductive organs. Sir Robert Jones, of St. Bartholomew's Hospital, London, states that 2% of the army had venereal diseases; and that out of 800,000 infants born in England and Wales, 100,000 die before the end of the first year; the largest number of these deaths were caused by venereal diseases.

Venereal subjects travel restricted in the United States. An amendment to the Interstate quarantine regulations announced by the Surgeon-General of the United States Public Health Service, 1919, reads: "All persons having venereal diseases must obtain a permit in writing from the local health officer before they will be permitted to engage in interstate travel. This permit must state that such travel is not dangerous to the public health.

In 1918 Congress appropriated one million dollars a year for two years, beginning July 1, 1918, to fight venereal disease. All these diseases must be reported. There is a heavy penalty for failure. They are to be investigated and the control studied.

Feeble-mindedness and insanity can frequently be traced to these diseases. The January, 1919, report of the Kansas Commission on Provision for the Feeble-minded, states that there are 7,500 feeble-minded persons in Kansas and less than one-tenth are cared for at the institution at Winfield. There were at least 1,500 feeble-minded children in the schools.

The highest cost per capita for maintenance of insane patients for 1919 was reported by the State of Maine: $306.97 and the lowest by Virginia, $135.90.

34% of 13,648 soldiers abstained from sexual intercourse. There was 60% more disease reported after the armistice was signed than before, probably due to lax regulations. 27% of 614 men who had acquired syphilis asserted they had intercourse without money.

It is due to the anti-venereal campaign, the character of the men, religion, loyalty to their wives and sweethearts, or to their self-respect, or perhaps resolutions before leaving this country, that the toll of venereal disease has been so low in our army.

"The United Presbyterian" states that there are 2,400,000 blind people in the world. Egypt leads with 1,325 per 100,000 population; India has 600,000; China, 500,000; Japan, 100,000, and the United States 100,000. In a recent year England had 174,000 insane people, of which 93,000 were women. Ireland had 24,000 and Scotland, 17,000. In 24 years 90,000 insane were admitted to the hospital in Paris. The United States has over 400,000 insane and feeble-minded people. Massachusetts heads the list with 344 for every 100,000. The number of insane is doubled every 16 years; and if the present rate continues to 2301, there will not be a single sane person in the United States. Five hundred million people sleep on dirt floors and are subject to attacks of vermin, etc. In India in the last 25 years over twenty million have starved to death and 1,400,000 children die of starvation and disease yearly.

New Jersey Eugenic Law, 1919. A certificate of health is a pre-requisite to matrimony. The purpose is to prevent the marriage of persons who are afflicted with contagious or social diseases.

Michigan has had such a law for several years, and beginning November 2, 1917, appropriated money for the tabulating of prostitutes and the control of these diseases. Eight hospitals have been established for the care of these patients. The patient must go to one of these special hospitals or his house will be placarded and he will be quarantined.

The Second Report of the Provost Marshal General up to December 20, 1918, states that 1.3% of the men examined for the United States Army were rejected on account of incapability due to these diseases.

On January 10, 1920, there were in the United States 62,683 insane single men and 26,047 insane married men, 37,115 insane single women and 35,975 insane married women.

It was found in an examination of prostitutes that 19% had reached reading grade in the public schools, 6% had reached high school, and 0.08% had one year in college. And it was also found that 20% were between 15 and 17 years of age, 29% between 18 and 20, 24% between 21 and 23,

10% between 24 and 26, 8% between 27 and 29, 2% between 30 and 32, 1% between 33 and 35, 1% between 36 and 38, and 1% over 38 years of age .

Back to School Campaign. Educating the child helps to eliminate these diseases. The Children's Bureau Bulletin states that there are 10 states which require an attendance of less than five months. In Alabama and Florida the children must attend 80 days; in Georgia, North and South Carolina, four months; Mississippi, 60 days; Tennessee, outside of cities of 5,000, 80 days; Texas, 100 days; Utah, outside of first and second class cities, 20 weeks; Virginia, 16 weeks; Delaware, 5 months.

GONORRHOEA—CLAP

An acute infectious disease of man only, and acquired by direct contact. It is one of the most widely distributed of all diseases among all classes of society and is the most common cause of pelvic diseases in women, necessitating the removal of the reproductive organs and causing sterility in both sexes.

"Ein kind; dann sterilitat."

"Once a gonorrhoeic, always"—no cure.

"More deadly than syphilis to women."

Blindness follows a conjunctivitis in the adult and in the babe shortly after birth and also later in life by the conveyance of pus to the eyes. Usually one eye is infected and the infection spreads, causing blindness in both eyes.

Authorities state: This disease causes 96% sterility in men, 99% of all pelvic operations in women. 90% of all babes born with seeing eyes, who become blind shortly after birth can blame gonorrhoeal parents. At least one-half of the total cases of blindness are caused by this disease. In one year in the United States, 12,000 babies went blind from this disease, acquired from parents who thought themselves cured before marriage. In nearly every case it was the father who had the infection.

99% of all cases are followed at some time, it may be years after infection, by complications, as rheumatism endocarditis, orchitis, pleurisy, iritis, abscesses, and many other manifestations; few if any cases are ever permanently cured, and few if any cases escape the after-effects.

In 1914 there were 6,000,000 people who had venereal diseases.

Many cities require the reporting of all venereal diseases, just the same as for smallpox and diphtheria. This disease should be reported to the Board of Health within 24 hours, or both the physician and patient should suffer a severe penalty. Such a law will become necessary in all the states before long.

Gonorrhoea is called the "Black or Hidden Plague."

Many states have passed marriage laws, ranging from an oath that the parties are free from disease, to a rigid examination by three competent physicians. This law not only includes venereal diseases, but epileptics, imbeciles, feeble-minded, common drunkards and consumptives. These laws are for the betterment of the people and prevent much suffering later in life. Laws have been passed in Michigan, Wisconsin, North Dakota, Colorado, Oklahoma, Utah, Wash-

ington, Indiana and Pennsylvania—very lax,—others are rapidly following, and the laws are becoming more rigid.

ANYONE WITH, OR EVER HAVING HAD GONNORRHOEA SHOULD NEVER MARRY.

This is one of the diseases which surely will show later in life. Even if the discharge is stopped, the disease is still there and may remain dormant for 25 years or more before manifestations appear.

Laws for the sterilization of criminals have been enacted in Indiana, California, Washington, Connecticut, New York, New Jersey, Iowa and Nevada, and others must soon follow.

In many of the states laws have been passed against mid-wives. They are fortunately passing out; on account of their ignorance, they were the cause of much blindness in babes.

Gonorrhoea, unlike Syphilis, is rarely if ever acquired innocently, and if not properly treated but permitted to run its course is followed by a continuous irritation and discharge—Gleet, an incurable disease. The discharge of both of these diseases may easily be stopped, but the organisms causing the diseases are in the body just the same. They are harboured in the prostate gland and other organs during the life of the infected patient.

The organism causing this disease is a roll-shaped diplococcus, non-motile, and was first isolated by Neisser in 878, and was given the name of Micrococcus Gonococcus. It is non-sporing (which is fortunate, or everyone would have the disease through carelessness of the infected), and grows on human blood agar as a grey-white, ill-defined edged, smooth, moist film on the surface of the medium. There is no growth on nutrien gelatin but a scanty growth on acid nutrient agar. It is hard to cultivate artificially. It grows at 37 degrees C., and at a maximum of 58 degrees C., and a minimum of 25 degrees C. It is both anaerobic and aerobic, reproducing by binary fission. In young cultures it may appear as aggregates.

The disease is not transmissible to the lower animals except when the pus is introduced into the eyes or testicles, thus fulfilling Koch's laws.

The Micrococcus Gonococcus appears microscopically coffee-bean shaped, and encapsulated about 5 by 15 microns, and is found in gonorrhoeal pus, (of which about 20% is phagocytes), both in and outside the leukocytes; also in glands and tissues, being carried by the blood to all parts of the body and to all organs. It will lie dormant in the

prostate for the whole life of the patient, and upon irritation, as drinking to excess, excessive meat eating, strains as heavy lifting, etc., will appear in the newly started discharge. It produces little or no toxin. The irritation is caused by the diplococcus breaking up into ultramicroscopical particles which invade the cells and tissues, and this irritation causes the symptoms of the disease. The disease is incurable. The fact is that the diplococcus lies dormant in the prostrate for an indefinite period and that the discharge has stopped is no sign that the patient is permanently cured, but to the sorrow of the unfortunate, it comes back in later years. For the first 20 days of the disease the diplococcus is found free and phagocitized in the pus; but after this time the diplococcus invades the deeper tissues and the discharge changes from a cream color to a greenish yellow, and the organisms are found almost entirely in the leukocytes and epithelial cells of the discharge.

The diplococcus grows readily on Loeffler's medium (horse serum may be substituted for human), the water of condensation only is inoculated; and after incubating at 37 degrees C., for 48 hours it appears as creeping transparent, glistening, colorless lines above the water of condensation and slowly ascending the surface of the slanted medium.

EXERCISE

1. Spread the pus from the discharge with the platinum needle on the cover glass or slide, air dry and fix in the flame.
2. Stain by Gram stain; it is Gram negative.
3. Stain another cover or slide by the special stain as instructed.
4. Stain another cover or slide with the capsule stain—30 seconds in a saturated acqueous solution of bichloride of mercury, wash in water, stain for 30 seconds with this solution: Saturated aqueous solution of methylene blue 1 mil, saturated aqueous solution of sodium carbonate 1 mil, and distilled water q. s. 100 mils. Wash in water and examine.

Urine examination. It is difficult to detect the diplococcus in urine. The patient should hold the urine for three hours after rising in the morning; the whole urine is centrifuged, and the precipitate then examined.

If there is anyone who has had this disease and believes himself cured, let him have a large dose of the Gonorrheal bacterial vaccine injected, made from a number of strains or

polyvalent; and if this will not start the discharge have the prostate massaged within 24 hours and he will find the diplicoccus is still with him.

Gonorrhoeal vaccines have not proven a cure but are of some use in the chronic form of the disease.

EXAMINATION FOR DISCONTINUING TREATMENT FOR MALES

1. Suspend treatment for one week. Give provocative stimulant alcohol in some form.
2. Retain urine over night, patient placed in position, meatus examined, urethra milked up from bulb, film and culture made.
3. Smear and culture from platinum loop scraping of urethral canal.
4. Micturate in three vessels, culture and film from centrifuged portion of the three vessels; thus the first, middle and last urine is examined.
5. Shreds or threads—film and also culture.
6. Cleanse glans penis with soap and sterile water; foreskin washed and urethra washed out with sterile water.
7. Patient in elbow-knee position, last drop of fluid shaken out of the urethra, the prostate and vesicles are palpitated, and massaged until a drop presents itself at the meatus; this drop may be opalescent and homogeneous, or in marked cases turbid, yellow and granular; a glycerin-like drop is produced from the anterior urethra, and shows that the prostatic or vesicular secretion has not presented itself at the meatus. Film and cultivate.
8. Urethral examination with the cystoscope for strictures, warts, follicles and abnormal conditions.

Only after all has been found negative may treatment be discontinued.

EXAMINATION FOR DISCONTINUING TREATMENT FOR FEMALES

1. The urine is cultured and filmed.
2. Examination for discharges; note quantity, consistency and color.
3. Vulva. Cleanse, and examine Bartholin's glands; note redness, et al. Contents filmed and cultured.
4. Urethra. Examine meatus for pus, examine floor;

culture and film; also film and culture from mucous membrane. Skene's tubes are also examined.

5. Vagina. Expose cervis, note erosion, polypi, cysts, et al., also secretions; film and culture.

Carry out this examination two times at intervals of one month.

There is no antitoxin for this disease, and there are very few antibodies formed. There is NO immunity of any kind.

THE COMPLEMENT FIXATION TEST

This test is applicable to many other micro-organisms besides the Diplococcus Conococcus.

1. The patient's serum. 5 mils of blood are taken aseptically from the vein in the arm. It is centrifuged to remove all cells; the serum is then inactivated in the water bath at 55 degrees C. for 30 minutes. It is tested for the presence of natural anti-sheep amboceptors, using for the test dose 0.2 mils of the inactivated serum with the test dose of the complement of 0.2 mils and the sheep erythrocytes 0.2 mils. If anti-sheep amboceptors are present and cause more than a trace of hemolysis they must be removed by adding to the serum 0.6% of the undiluted washed sheep corpuscles, placing in a vessel surrounded by cracked ice for 30 minutes and then remove the cells by centrifuging. It has been found that 66% of the serums require the removal of the anti-sheep amboceptors.

2. The antigen. As many as possible should be used, at least 25 or 30 different strains should be obtained; these are grown on hydrocele agar (for Micrococcus Gonococcus) for four days (other organisms to be grown on media best suited for growth), washed with sterile water and centrifuged. The sediment must be repeatedly washed and centrifuged. To the washed sediment add 0.85% normal sterile salt solution, in the proportion of 100 to 1, place in a glass container with glass beads and shake for 24 hours, then incubate and autolize for 48 hours at 37 degrees C. Then filter through coarse paper to remove the glass beads. To the filtrate add 0.3% tricresol and place in sterile brown or amber glass containers; and keep in the refrigerator for two weeks; at the end of this time sterility tests are made. For use it is diluted with 9 parts of an 0.85% normal sterile salt solution.

3. Anti-complimentary Value. Increasing amounts of the antigen are diluted 1 in 10 and are added to 0.2 mils of

inactivated normal human serum, 0.2 mils of the complement and a 1 in 10 normal salt solution sufficient to make 2 mils. This is incubated at 38 degrees for 30 minutes, then 1 unit each of the amboceptor and sheep erythrocytes (0.2 mil) are added and again incubated for 30 minutes. This will indicate the largest amount of antigen which will not inhibit hemolysis under exact test conditions.

4. Antigenic or fixing value. Increasing amounts of dilute 1 in 10 antigen are added to 0.2 mils inactivated serum obtained from a patient with the disease viz: for Gonorrhoea—a patient with chronic gonorrhoeal arthritis—and a similar process as in No. 3 is carried out,—indicating the smallest amount of the antigen which will fix the complement and inhibit hemolysis in the presence of a positive serum under exact test conditions.

5. The Hemolytic Serum. Fresh guinea pig serum is diluted 1 to 10 with normal salt solution and serves as the complement in 0.2 mil amounts. Sheep corpuscles are washed three times with normal salt solution and made into a 5% suspension and used in 0.2 mil doses. The anti-sheep amboceptor is diluted so that 0.1 mil contains one hemolytic unit. Titrate the amboceptor against 0.2 mils of the serum to be tested and the above amount of the complement—the total amount made up to 2 mils with saline solution. One hemolytic unit is used in the test.

The test—

1. Prepare the complement and dilute 1 in 10.
2. Prepare the corpuscle suspension and dilute 1 in 10.
3. Prepare patient's serum, inactivate at 55 degrees C. for 30 minutes.
4. Determine if patient's serum contains natural anti-sheep hemolysin.
5. Titrate the anti-sheep amboceptor against the patient's serum. Dilute so that 0.3 mils contains 1 unit.
6. Dilute the antigen 1 in 10.
7. Use controls. Place the serum, antigen and complement mixture and control in the water bath for 30 minutes at 38 degrees C.; then add the amboceptor and erythrocytes. Again place in the water bath until the serum and antigen controls are hemolyzed. Immediately remove, and make readings.

The disease called big pox, distinguished it from small-pox. It is the greatest of the hidden plagues. It was described by Chinese physicians 200 B. C., but was not recognized as Syphilis until near the end of the 15th century; although the Bible speaks of a disease as being handed down to the third and fourth generation of those that sinned, which probably was syphilis. The Bible also speaks of "emeroids of the secret parts," the "man with the running issue," and the "itch whereof thou canst not be healed." "The daughters of Moab vexed the Israelites with their wiles." Syphilis has been a skeleton in the closet of every member of the so-called religious cults of Rome, Assyria and Asia Minor, whose teachings were of the wildest licenses and the holy meetings sexual debauches.

Syphilis became pandemic about the end of the 15th century, soon after the soldiers of King Charles VII, reached Naples. It became so prevalent that Astruc said, "Everyone in Christendom from the Pope to the lowest scullion was infected." Ever since the mark of syphilis has been left upon some door in every community. Charles' triumphant entry into Italy turned to a retreat of broken and diseased soldiers a little less than one year (1495) later. These scattering diseased soldiers carried the disease through all Europe, syphilis appeared in Switzerland in 1495, Greece a year later, and England and Scotland in 1497, and Russia and Hungary in 1499, as epidemics.

In 1496, it had become so prevalent in Paris that parliament decreed that all afflicted persons should leave the city within 24 hours.

In 1496 and 1497, Nurnberg attempted prophylactic measures, and April 21, 1497, Aberdeen, Scotland, ordered all light women to desist from their son or be branded on the cheek with a hot iron and be banished from the city. Six months later Edenburg ordered all affected to be banished to the island, Inchketch, near Leeth.

So it is seen that in a very short time the disease spread to be a world-wide epidemic. Probably it was first carried to Europe by Columbus and his party, it being acquired from the Haiti Indian women. In the broad trail of diseased bones, it has been found in excavations; it has been traced from South and Central America to North America and also from the West Indies and Haiti to North America and Europe.

Up to the year 1600, prostitution and the army were not

connected. Strozze in 1570 caused over 800 prostitute army followers to be drowned in the river Lovire. In 1648 during the 30 year war, rations were issued to 40,000 soldiers and to 140,000 camp followers not on the ration list. These women cooked, washed, mended clothes and nursed the wounded. In 1608 Johann substituted men for nurses and venereal disease fell off. Cornor, of the French army, drove away 3,000 women followers and said they caused one hundred times more harm than the enemies' guns.

Syphilis is acquired by infection, contact or inheritance. Sometimes it is innocently acquired as in the case of physicians and dentists in their practice with syphilitics. About 1% of all cases are acquired innocently.

Syphilitic infection may be divided into:

1. Acquired syphilis.
    a. Gummata.
    b. White pneumonia.
    c. Fibroid indurata.
    d. Syphilitic phthisis.
2. Inherited syphilis.
    a. Circumscribed gumma.
    b. Diffuse-interstitial.

Syphilis is considered the third killing disease and is caused by the Treponema Pallidum (Spirochaeta Pallidum), which was isolated by Lustgarten in 1893. Metchnikoff and Roux transmitted the disease to the higher apes; and in 1905 it was discovered that the virus was not filterable. Noguchi has grown six strains of the organism artificially, three of which produced syphilis in rabbit testicles and one sub-strain was cultivated to the 25th generation. The organism is 4 to 20 by 0.5 microns, and is a closed spiral, with regular 3 to 12 curves and has a forward as well as the rolling motion. Syphilis is an infectious disease, acquired by sexual congress, or transmitted by a diseased person; and may also be transmitted by the bites of bed bugs, lice and other vermin, towels, wash basins, smoking pipes, et al. However it is usually transmitted by contact.

There are three stages to the disease:

1. Chancre. In about 15 days after contact, a pimple appears at the point of contact—it is hard and gradually grows larger, disappearing in about 10 to 20 days.

2. In 30 to 90 days after contact, spots or small pimples appear on the arms, abdomen, toes, back and hair line, sometimes entirely covering the whole body—whitish casts

or patches in the mouth and throat, which may last from 6 to 24 months. These pustules leave the permanent pits or marks. This stage is very infectious, and Salversan, or 606, is of great benefit in causing these pustules to disappear.

3. This stage lasts the whole lifetime of the patient,—the life is usually shortened; the disease goes inward to the bones, affects various organs and the brain; a paralysis sets in, caused by the rotting of the body parts. 95% of all locomotor ataxia is caused by this stage of the disease.

There is a cure. By taking the old-line mercury treatment faithfully for five years. Salversan is useful in clearing up the pustules in the secondary stage; but as for its being a cure, it is too soon to say. We must wait until the third and fourth generations before it is pronounced a cure. Salversan and its improvements are very dangerous to use. They have caused many deaths; but a patient with venereal disease is probably just as well off dead. The pits or scars left after the second stage are always to be seen. They are just as plain and larger than the smallpox pits; hence the name for this disease—big pox.

Chanchroid—soft chancre or bubos, blue balls—is not syphilis but a different disease. It appears as soft eating ulcers, appearing from one to two hours after contact at the exposed part. It is very infectious. The lymphatic glands swell, especially those of the groin, on account of being filled with pus, containing the bacillus causing the disease, very infectious. The bacillus produces a toxin which spreads, If this is not lanced, suppuration takes place. The pus is rapidly to all the lymph channels and by them to all parts of the body.

DIFFERENTIATION OF THE TREPONEMA PALLIDUM

ORGANISM	MOVEMENT	Attack Tissue	Putrid Odor	Pathogenic	CURVES
Pallidum	Rotary	Will	No	Is	Many dense, regular
icterohemorrhagiae	Rotary	Not	Has	Not	4 to 6, faint, many
microdentum	Vibratory	Will	No	Not	dense, regular
macrodentum	Wavy, serpentine	Not	No	Not	3 to 14, less regular
refrigens					3 to 8, faint, irregular, two times as wide as the erythrocyte

Syphilis is more deadly to men than to women, while gonorrhoea causes more deaths in women than men. Dr Leredde, of Paris, states that there were between 200,000 and 300,000 persons who contracted syphilis during the war and that three-fourths remain actively syphiletic and spread the disease. Dr. Clement Simon states that during 1916 there were reported 45,000 army cases and says that probably there were as many more unreported cases.

Syphilis is the cause of many idiots—those having the the mentality of a normal child of seven years; and morons—those having the mentality of a child of 2 years; and is the cause of much of the babe mortality, which occurred in 1916 to the extent of 110 per 1,000 in England; 92 per 1,000 in Ireland; and 126 per 1,000 in Scotland; and in the cities of the United States in 1915 as follows: New York 98, Boston 104, Buffalo 108, Chicago 114, Cleveland 110, Denver 131, Detroit 104, Louisville 113, New Orleans 120, Philadelphia 105, Pittsburgh 110, Washington, D. C., 110, Rochester 84, St. Louis 82, Indianapolis 89, Los Angeles 67, Seattle 53.

Syphilis costs the state of New York yearly 35 million dollars; one institution contains 20% insane males due to syphilis and 8% females due to it.

The British General Hospital reports for January 1, 1915, that it treated 11,500 syphilitics, giving 89,000 injections of salversan.

In 44,148 pregnancies of syphilitics, 3% of the foetus died before term, 30% died in early age and only 1% were normal. Jeans, of St. Louis, states that 15% of the males examined had syphilis; and 10% of these men infected their wives, and of the births only 3.5% lived. Pierce, of Washington, examined 2,933 prostitutes and found only one not infected, and that of the free clinics provided there were 25,224 patients in 1918, an average of 41.09 patients daily.

To cultivate Treponema Pallidum.

Watabiki's medium. 200 mils cow's milk warmed to 60 degrees C., and 590 mils ascitic agar are added drop by drop, the milk being shaken to cause precipitation of the casein.—Filtered through paper, then 10% NaOH added to the filtrate, until slightly alkaline. Then 2 grams of urea is added and the whole sterilized at 60 degrees C., for 30 minutes a day for three days. One part of serum or blood is added to two parts of this medium.

—Or inoculations into the testes of a rabbit and examined 30 days later.

—Or tissues from a living paretic brain macerated in normal soline solution, and planted deep in tubes of rabbit kidney ascitic agar and covered with paraffin oil to make anaerobic, and incubated four weeks—sub cultures can be successfully grown.

—Or serum medium—equal parts sheep, horse and rabbit serum and three parts distilled water to which a piece of sterile rabbit tissue has been added, and grown anaerobically. After the first growth in this liquid, sub-cultures may be made on solid media.

Staining.

Giemsa's stain. Must be made as used. 12 mils of the eosin solution (2.5 mils of 1% eosin in 500 mils water), 3 mils of a 1 in 1,000 aqueous solution of No. 1 azure, and 3 mils of a 0.8 in 1,000 solution of azure No. 2.

The films are air-dried, hardened in absolute alcohol and stained for 24 hours, washed in water, dried and examined. The spirilla are a pale rose color.

Or India ink. A drop of the fluid from the lesion is mixed with a drop of Chin-Chin liquid pearl ink on a slide, air-dried and examined,—the organisms as seen against a dark background of carbon particles.

Or. The Silver stain. Fragments of syphilitic organs are fixed for 24 hours in 10% formalin; washed in 96% alcohol for 15 hours; washed with distilled water until the fragments no longer float; and impregnated for 5 hours in 1% silver nitrate solution 1 mil and pyridin solution (10 in 100 parts) 1 mil; then washed in a 10% pyridin solution and reduced by immersing for 2 hours in 4% pyrogallic acid, purified acetone 10 in 100, and 15 parts per total volume of pyridin added at the time of using.

Dark field examination. Cleanse the lesion, take specimen, place on a thin slide, cover with a thin cover-glass, place a drop of immersion oil on the cover glass, and another drop on the top of the condenser, place slide on the stage and focus, by adusting the mirror and by looking through the microscope with the low power lens in place; focusing up and down, until the concentric ring becomes a point in the center of the specimen. Replace the low power lens with the oil immersion lens. If the light is not sufficient, a bull's-eye may be made by filling a 2 liter flask with water and placing it between the light and the mirror.

THE WASSERMAN REACTION

A test to determine if the syphilitic organism is present in the system of the patient. The reaction is due to certain

bodies in the blood serum, which display marked affinity fo lipoids and lecithin. An emulsion of lecithin or guinea pi heart may be used instead of the antigen which later is ob tained from livers of syphilitic fetus.

1. The antigen.

   a. The liver of a syphilitic fetus is cut in smal pieces and an emulsion is made by using live 1 gram, saline solution 5 mils. The superna tant liquid is removed centrifuged and ½% phenol added and stored on ice until used.

   b. If lecithen is used as an antigen, a solution o pure lecithin in alcohol is made, using 0.1 gram to 100 mils saline solution, and stored on ice

   c. Guinea pig heart extract; the heart is rubbed very fine in a mortar with powdered glass and alcohol using 1 gram of heart to 25 mils of absolute alcohol. It is then heated for one hour at 60 degrees C, filtered and stored on ice.

The strength of the antigen varies in different preparations, and it is standardized so that it is of such strength that the quantity will not hemolize 1 mil of a 5% solution of washed lamb's blood corpuscles in the presence of 0.2 mil of a known positive serum, 0.1 mil of the complement and 2 initial units of the hemolytic serum. Determine the unit as follows: A series of test tubes is prepared, each containing the same amount of the above named reagents and varying amounts of the antigen. The usual technic is followed and the unit determined by the quantity of the antigen that inhibits hemolysis. Then this same antigen must be tested with a known serum used in place of the positive serum, using double the amounts or units of blood cells. The unit having been determined, the antigen is so diluted that 1 mil will contain 1 unit.

2. The antibody. The blood serum or cerebrospinal serum of a syphilitic person is collected, centrifuged and the clear serum only is used.

3. Complement. The normal guinea pig blood is obtained; it must be fresh, as it loses its complementary value if kept longer than 24 hours. It is defibrinated, centrifuged and stored on ice, and kept frozen.

4. Hemolytic serum. Serum of a rabbit which had been injected with washed lamb's blood corpuscles. Immunize the rabbit by injecting 5 mils of washed lamb's blood corpuscles to which has been added 15 mils of sodium citrate solution to prevent clotting,—inject at intervals of 5 days or 6 in-

jections. Ten days after the last injection, test the rabbit's blood by determining the minimum amount of the serum which will hemolize 1 mil of a 5% suspension of washed lamb's blood corpuscles with 0.1 mil of normal guinea pig complement. Various amounts are added to test tubes with 1 mil of the suspension of lamb's blood corpuscles and 0.1 mil of the complement; incubated at 37 degrees C., for 30 minutes; examined to determine the smallest quantity of serum which produces hemolysis—usually 1 mil of a 1 in 2,000 dilution in normal saline; and the quantity necessary for the dilution is, as a minmum, 2 units, thus 1 mil of a 1 in 1,000 dilution is used for the reaction. The dilution must not be under 1 in 1,000; if this is the case the rabbit must be given more injections. The blood having been tested is centrifuged and repeatedly washed with normal saline solution, then mixed with a 1% sodium citrate solution.

5. Lamb's blood corpuscles. 5 mils of defibrinated lamb's blood, collected and washed. A 5% suspension is made in normal saline solution.

The antigen, antibody (patient's serum) complement and hemolytic serums must each be diluted with normal saline solution, so that 1 mil of the dilution will contain the necessary quantities as needed for the reaction.

The antigen and the hemolytic serum must be inactivated to destroy the complement before using, by heating at 56 degrees C., for 45 minutes. This must be done as soon as made.

The reaction.

Place in a test tube 0.2 mils of the antigen and add 1 mil of the patient's serum, or the antibody, and 1 mil of the complement; incubate 45 minutes at 37 degrees C.; then add 1 mil of the hemolytic serum containing 2 minimal doses and 1 mil of the 5% suspension of lamb's blood corpuscles; again incubate for 2 hours; place on ice over night if hemolysis has not occurred. If the antibody of syphilis is not present in the suspected blood serum, hemmolysis will not occur on account of the complement being fixed to the immune body and the reaction is positive. Should the suspected blood serum not contain the specific antibody, hemolysis will occur because there is no immune body to fix the complement, therefore causing hemolytic amboceptor or hemolytic serum, by the aid of the red blood corpuscles, to fix the complement, producing hemolysis, and the reaction is negative. Controls must always be carried, for the reagents are subject to external influence, which will greatly vary their action.

THE COMPLEMENT FIXATION TEST

Is carried out as given under Gonorrhoea.

THE HECHT-WEINBERG-GRADWOHL MODIFICATION OF THE WASSERMAN

Place in a rack 14 small test tubes. The first 10 of these tubes are used to determine the hemolytic index of the suspected blood or the exact amount of hemolytic amboceptor present in the given blood serum. The last four tubes are used in the actual test. Add 0.1 c.c. of fresh unheated patient's blood serum to each of the first ten tubes. Then add decreasing amounts of normal salt solution to these tubes beginning with 1 c.c., then, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 and 0.1 c.c. to the succeeding nine tubes. Next add increasing amounts of fresh 5% suspensions of sheep's blood, starting with 0.1 c.c. and ending with 1 c.c. Place the rack in the water bath for one-half hour. The tube which last shows complete hemolysis constitutes our "hemolytic index"; if it is Tube 4, our index is 4, because this tube had received 0.4 c.c. of sheep's corpuscles to be added to the last four tubes. The first three tubes (11, 12 and 13) constitute the tubes for the actual test, while the last tube in the rack (Tube 14) serves as our serum control tube. Tubes 11, 12 and 13 receive, therefore, the patient's serum, the proper amount of sheep's corpuscles, dependent on hemolytic index, rising strengths of antigen, but no complement and no amboceptor. Tube 14 receives only sheep's corpuscles, but no antigen.

Use 0.1 c.c. of a diluted antigen, determined by titration in tube 11, 0.15 c.c. antigen in Tube 12, and 0.2 c.c. in Tube 13. In order to equalize the volume of fluid in all these tubes, add 0.2 c.c. normal saline to Tube 11, 0.15 c.c. to Tube 12, 0.1 c.c. to Tube 13, and 0.3 c.c. to Tube 14. The tubes are then agitated and placed in the water bath for half an hour. These last four tubes are filled at the same time the additions to the first ten and are left with them in the water bath for one-half hour for fixation of complement, the rack is then taken out and the hemolytic index computed. If the index is low, say from 1.4, add 0.1 c.c. of sheep's blood to the last four tubes. If the index is between 5 and 7, use 0.15 c.c. sheep's blood to the last four tubes; if between 10 and 15, use 0.25 c.c.; if between 15 and 18, use 0.3 c.c.; and if between 18 and 20, use 0.35 c.c. If the patient's serum has an index below 3, regard the reaction as of doubtful value. If it is above 3 it is absolute. The reaction is read

off exactly as in the Wasserman, that is, inhibition or non-inhibition of hemolysis. If the amount of complement or natural antisheep amboceptor is very low, add the proper amount of guinea pig's serum or rabbit's immune serum, ascertained by preliminary titration.

NOGUCHI MODIFICATION OF THE WASSERMAN

Serum of horse, sheep or rabbit is diluted 1 in 3 with water to which has been added a piece of sterilized rabbit testicle, the whole made slightly alkaline, sterilized and covered with a thick layer of paraffin oil, to make anaerobic. Material for this test is sold in dry form.

FOR CEREBROSPINAL FLUIDS

The fluid is not inactivated and large amounts must be used.

FOR MALIGNANT DISEASES

Antigen.—Malignant tissue cut very fine, extracted for two hours with 20 volumes of C. P. acetone, with constant shaking, and filtered.—For use a portion is evaporated to dryness at 37 degrees C., redissolved in half the amount of absolute alcohol; and diluted with saline solution according to the result of the titer. As much saline may be used providing twice as much is not anti-complementary.

The blood serum.—10 mils of blood (best taken from a paretic) is mixed with 0.1 mil of a 2% sodium oxalate solution, the cells washed three times with saline solution, centrifuged and weighed. For each part by weight add 20 volumes of C. P. acetone. Extract for three days with frequent shakings, then filter and evaporate in a tarred vessel in the incubator; then add 95% alcohol to make a 1% solution.

The Test.—Mix 1 part of this solution with 4 parts saline solution and titrate; usually 0.8 mil is required. The serum to be tested separated from the clot, is cooled in the ice box and 2 volumes NaOH solution is added. The complement serum is 1 mil of a 5% hemolytic sheep or beef serum. Add to a series of test tubes 0.6, 0.3, 0.12 and 0.75 mils of the inactivated serum; then add to each tube 1 mil of a 5% complement and 0.8 mils antigen; bring all tubes to the same volume; mix and stand 3 hours at room temperature; then add to each tube 2 units of amboceptor (1 unit is the smallest amount which completely hemolizes 1 mil of 5% red blood cell emulsion in 2 hours in the presence of 1 mil of a 5% complement and 1 mil of a 5% red blood cell emulsion) and in three hours read results.

FOR TUBERCULOSIS

Antigen—Mixture of Koch's old tuberculin and an extract of tubercular granulating tissue freed from all other tissue. Prepare the tissue with 4 parts alcohol and extract for five days, filter and dilute with Sparts saline solution, and test against the patient's serum. Add 9 volumes of diluted extract to 1 volume old tuberculin and titrate. The Test: 1 mil each of the complement serum and antigen is placed in test tubes; then add 0.1 mil inactivated serum; bring all tubes to the same volume; stand 3 hours at room temperature; thern add to each tube 2 units amboceptor and 1 mil of a 5% red blood cell emulsion; incubate 1 hour.

ABDERHALDEN TEST—OR ABDERHALDEN'S DIALYZATION TEST

Tissue of a known disease when mixed with blood serum of the patient suffering from the same disease will cause dialysis; as cancer tissue and serum from a patient suffering from cancer will when mixed produce dialysis. When foreign proteins, fats or carbohydrates are injected into the circulation, ferments are produced, which are able to digest the injected material; these ferments are specific acting only upon the substance, the presence of which has led to their appearance in the blood. The Reagents: Tissue is cut in small pieces and extracted in the Soxhlet apparatus; washed in saline solution; squeezed dry and ground in a mortar; washed in running water; then washed in water to which has been added 5 drops of acetic acid to the liter and the whole boiled for 10 minutes. The tissue is placed on a sieve and boiled in 5 volumes of water; this is repeated 6 times. 5 mils of this solution is added to 1 mil of a 1% ninhydrin solution and boiled for 1 minute, when it should show no violet color after standing 30 minutes and the tissue should be white; if it is not, it must be boiled in water for 5 minutes and washed 5 minutes, alternatively, until no reaction is obtained. Preserve in sterile water covered with chloroform and toluol. The patient's blood is obtained by taking 15 to 20 mils from the vein before breakfast—it must be taken during fasting. Coagulate and settle for 5 hours in the ice chest; centrifuge for 10 minutes. Serum over 12 hours cannot be used. Use a Schliches dialyzer shell, which has previously been tested to impermeability of albumen and silk peptone. Absolute cleanliness is necessary; all vessels must be sterile and the test carried out in a special room.

The Test. With sterile forceps remove the amount of tissue substrate needed; cover with 5 volumes water and boil for 5 minutes. Test 5 mils of the filtrate with 1 mil of a 1% ninhydrin as previously directed; if a trace of color is visible after boiling for 1 minute and standing for 30 minutes the substrate must not be used. Place into a proved shell in a dry Ehrlenmeyer flask and add 0.5 grams of the substrate, crushing and tearing to increase the surface area of the tissue. One shell is used for each serum to be tested, and place one shell for the control into which no subtrate is added. To each shell add 1.5 mils of serum; wash the shells with 20 mils of sterile water, and transfer to new flasks, each containing 20 mils of sterile water covered with tuluol; and incubate at 37 degrees C., for 16 hours, when on removal the toluol must be inside as well as outside the shell. 10 mils of the dialysis is now placed in large dry test tubes and to each test tube is added 0.2 mils of a 1% ninhydrin solution; place in each test tube a bumping rod, and boil for 1 minute and stand for 30 minutes; examine for color; if the serum or control gives no color while the serum plus the substrate shows even the faintest color, the test is positive. When the dialysate of the serum alone shows colors due perhaps to hemoglobin or digestive serum products, the reaction is positive, providing the serum plus the substrate shows a darker color. This reaction is difficult and may be performed only by the experienced laboratory worker.

BRUCK'S SEROCHEMICAL TEST

Nitric acid when added to syphilitic blood serum causes a precipitate to form. Bruck's technic is based on an acid containing, per hundred gm. 24.77 gm. of nitric acid, or, per hundred c.c. of nitric acid with a specific gravity of 1,149. He uses 0.3 c.c. to 0.5 c.c. of clear serum, 2 c.c. of distilled water are added. This is shaken, and then 0.3 c.c. of nitric acid is added with a standardized pipet This is shaken and permitted to stand at room temperature for ten minutes. Then 16 c.c. of distilled water at 15 degrees C. are added and shaken slowly three or four times so as not to foam. This shaking is repeated ten minutes later, and then the tube is set aside for one-half hour. If the serum is syphilitic it shows a distinct flocculent turbidity. In 12 hours, a precipitate is piled up on the floor of the test tube. If the serum is non-syphilitic, there is no precipitate at any time.

LANDAU COLOR TEST

To 0.2 mils of the suspected blood serum, which has been heated to 56 degrees C., for 30 minutes, add 0.1 mil of the reagent consisting of a 1% solution of iodine in carbon tetrachloride; shade thoroughly until all iodine color has disappeared; set aside for 5 hours at room temperature. A positive reaction is indicated by a clear transparent yellow, negative by opaque whitish gray mixture. Confirm the test by adding 2 drops of starch paste; and if any unbound iodine is present, a blue color is produced which indicates a negative test; also confirm by adding 0.2 mils of ammonia water. The serum must not be over 10 hours old. If no blue color appears after adding the starch paste, the reaction is positive.

PALLIDIN TEST

1 mil of ground extract of human syphilitic organisms heated to 60 degrees C., for 30 minutes, and to which 0.5% phenol has been added, is used as the reagent. The upper arm is cleansed, the skin scarified and the reagent is rubbed in. In 48 hours a dry red papulose eruption appears covering a space of about ½ cm. This means a positive reaction, which is obtained usually in inherited or tertiary syphilis. A faint reaction never occurs in primary or secondary stages. Negative tests show no reaction.

LUETIN REACTION

Ground cultures of many strains of the organism are diluted to a fixed strength, killed by heat and 5% phenol added. It is tested by culture and animals for sterility. 0.05 mills are injected hypodermically in the upper arm; and the same amount of water and 5% phenol also injected nearby as a control. A positive reaction is indicated by a small raised area or pus tubule at the site of injection within 24 to 48 hours, and remaining for several days; or it may pustulate and discharge; the height is reached in four to five days; then it usually subsides. A temperature, maiaise, loss of appetite and diarrhoea may accompany the reaction. In the tertiary stage of syphilis a mild reaction is given, and also in the first and second stages, when the redness at the site of injection may fade in three to four days, and in ten or more days enlarge and form pustules. The same test may be applied for gonorrhoea using a glycerin extract of a number of strains of the diplococcus, which have been ground in an agate mortar, killed and preserved with 5% phenol.

SMALL PARASITE FROM SYPHILITIC LESION.

AMEBOID LEUKOCYTE. INCLUSION CELL FORM ATTACHED TO CHROMATIN.

GRANULES IN MOTION

DEEPLY STAINED GRANULES, ROUND, OVAL OR PEAR SHAPED OF A COPPER COLOR.

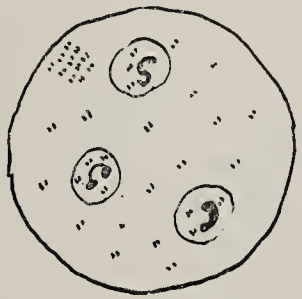
DIVISION OF CHROMATIN AND FORMING FREE FORMS.

THEORY OF REPRODUCTION OF TREPONEMA PALLIDIUM (THE SYPHILIS MICRO-ORGANISM.)

TREPONEMA PALLIDIUM OR SPIROCHAETA PALLIDIUM

CONFUSIN SPIROCHETES FROM NON SYPHILITICS.

SCRAPINGS FROM SYPHILITIC LESIONS.



GONORRHEAL PUSS.

TUBERCULOSIS AND CONSUMPTION

Tuberculosis is caused by the Bacillus tuberculosis, while Consumption, the consuming disease, is caused by the Bacillus tuberculosis and various streptococci and staphylococci. As in the case of an infection of the lungs by the Bacillus tuberculosis, nature causes a walling up of the bacillus in nodules or tubers, hence the name tuberculosis; but when a complex infection results by the invasion of the various streptococci and staphylococci, these organisms tear down the walls of the nodules, and a consuming of the tissue results, forming the large cavities which eventually will cause destrution of the entire lungs—hence the name Consumption.

Consumption is usually accompanied with anemia, leukocytosis and lymphocytosis.

Agglutinins (readily demonstrated in immunized goat blood) and precipitins are inconstant in spontaneous tuberculosis, but are demonstrated in experimental tuberculosis. Opsonins are reduced and are fluctuating. Complement-fixation bodies appear after injecting iver of dead bacilli.

The disease caused over one-tenth of all deaths, or 140,000, in the United States in 1913, and Kober estimates that the yearly loss is $214,000,000. Comparatively few people escape infection with the bacillus; about one in every eight deaths is caused by consumption. Many infected persons are cured and die of other diseases.

In 1914 in the United States it was estimated that there were 9,000,000 infected persons and $20,500,000.00 was spent for the patients' relief. 68.8% of this amount came from the states, cities and counties, the balance from private contributions.

In 1909, 47% of the money spent for the patients' relief came from private sources and New York State spent $3,200,000.00; Illinois, $1,700,000; Pennsylvania, $1,600,000; Massachusetts, $3,200,000, and California, $4,650,000. Deaths from this disease decreased 70% but those from cancer increased 4.51%.

We spend annually over $20,000,000 for prophylaxis of this preventable disease.

It is stated that 20% of the United States Army rejections in 1917 were on account of consumption. The draft boards, up to 1919, rejected 50,000 as consumptives; 12,000 developed consumption in the army.

Every 2½ minutes some person dies from consumption—548 every day or 200,000 every year. Consumption is the

ause of at least one-third of all deaths between the ages of 0 and 35—more in cities than in the country.

The Civil War killed 205,070—during this same time 00,000 died from consumption.

The Pennsylvania State Department of Health in their eport for 1919 state that there are 50,000 persons in this state, who are going about freely while consumption insidously gets a grip upon their bodies and menaces their families, their friends and their communities. Some of these 50,000 do not know that they have consumption because they have not consulted a physician or have not recognized the symptoms. The others fail to secure regular medical attention through timidity, fear or indifference. In many other diseases victims are promptly quarantined or separated from other people. Of the 50,000 persons so affected, many come and go at will as a direct menace to themselves and their associates.

There are at least 10,000 deaths annually in this state from consumption, and at least 75,000 others have the disease.

Pennsylvania has three large state sanatoria: Mt. Alto, Cresson and Hamburg. Each of these institutions has 2,000 beds and treats annually nearly 10,000 persons. City, county and private institutions probably have 1,500 beds and treats 3,000 patients yearly. Then about 7,000 persons having consumption will go to the mountains or receive regular medical treatment.

Allegheny county has an average yearly death of 1,500 consumptives and probably 10,000 are affected.

In 1919 the United States had a death rate from consumption of about 150,000, and these were mostly active men and women of the ages between 16 and 45. The war killed about 67,000; during this same period 200,000 died of consumption, with an economic waste of $500,000,000.

The organisms causing this disease are carried by the hands, food, flies, kissing, eating utensils and in many cases the disease is due to droplet infection—coughing in the open.

Reiche states that out of 6,000 consumptives examined, 66.5% gave no family history of consumption. Other authorities state that 90% of all children are infected with tuberculosis before they become 14 years of age. 84% of babes with intestinal consumptoin are fed on canned milk. 95% of all infections take place by inhalations of the Bacillus tuberculosis.

In France, before the war, one out of every four deaths was caused by consumption, and up to January, 1917, France has 500,000 soldiers infected with consumption.

A report dated January 5, 1918, states there were 5,000 cases of consumption in the Italian army and that 30,000 prisoners returned from Austria had contracted the disease.

In 1915, the National Association for the Study and Prevention of Tuberculosis reported that $22,500,000 was spent in anti-tuberculosis campaigns. $350,000 was spent in teaching and treating tuberculous children and $750,000 was spent in the cure of tuberculous insane and criminals.

In one institution, of the babes born of tuberculosis families, 40% had tubercular mothers; of these there were 334 breast-fed and 10.5% died; of the 124 bottle-fed, 28% died. Out of 124 which died during the first year of life, 37.1% died of consumption. The babe is born with a non-specific immunity, part of which is against consumption, but as the babe is in close and constant association with the tubercular mother for the first six or more months of its life, the mother throwing out billions of the bacilli (it is estimated that a consumptive will expectorate from 500,000,000 to 3,000,000,000 tubercle bacilli in 24 hours) at every cough, will readily infect the babe. Consumption is not inherited.

Practically all animals are susceptible to the disease. The cow is very susceptible, while the goat and hog are very resistant. Horses and sheep are rarely attacked. It is stated that from a fraction of 1% to 30% of the cattle slaughtered for food are tubercular, hogs 25%, sheep 1%.

An uncleanly consumptive may scatter broadcast billions of bacteria daily.

The cleanly consumptive is of practically no danger to others.

The usual source of pulmonary infection for man is from droplet infection or dried sputum, and the miliary (general) or intestinal is from milk or meat from tubercular cattle. Many cases can be avoided by keeping the hands away from the mouth and washing them well before eating.

The A. M. A. Journal December 18, 1920, reports an investigation completed by Dr. Rodgers of the Cincinnati Tuberculosis Sanatorium, which shows that Droplets, salvia, gauze used by the patients, pillow cases, urine, eating utensils, magazines, door knobs and patient's hands were some of the means of dissemination of the organisms of consumption. Dr. Rodgers made cultures from the above named sources and found that the organisms were present and could cause consumption. When guinea pigs were inoculated with particles from coughing patients, collected 15 inches

from the patient's face, 35% of the pigs died, when inoculated with particles collected 6 inches from the patient's face, 75% of the pigs died.

Rooms which are not properly aired and are sunless and windowless, favor the disease. Out of doors, sunlight, nourishing foods and complete rest are the best medicines. Flies carry the disease. Eating utensils as knives, etc., not properly washed, have caused whole families to acquire the disease. Meat and milk should be well cooked and boiled to prevent a possible infection. Repeated self-reinfection takes place by the consumptive swallowing his own sputum and this has a tendency to infect and cause intestinal and miliary consumption.

Every segregated case means at least three less deaths. Educate the people as well as the consumptive in personal hygiene, and there will be few cases of consumption.

There is no natural immunity to the disease. Mummies as far back as 3500 B. C. show signs of the disease. There is little or no artificial immunity.

There are three distinct types of bacilli causing tuberculosis—the human, bovine and avian types; all probably have come from a common ancestor, through adaptation to special hosts, or they may be of plastic habitat varieties, which under certain conditions transform from one into the other.

The human type. Firct demonstrated as an inoculable disease by Villemin in 1865; the bacillus was discovered by Baumgarten in 1882. It is a slender straight or curved rod, about 2 to 4 microns long and 0.3 to 0.5 microns wide, about half as long as a red blood cell is wide. It may occur singly, or end to end or grouped. It is a capsulated obligatory, non-sporing bacillus. It contains fat, albuminous and aromatic substances. In some cultures, a filamentous growth is seen, and in others clubbed ends or branching may occur; probably these are involuted forms. A beaded appearance is seen in the first and last stages of consumption probably due to the breaking down of the bacillus. This bacillus should be classed as a streptothrix one of the higher bacteria.

Beaded bacilli are also found in leprosy, with the leprosy bacillus, which is also acid fast. These beaded bacilli appear like a row of bright red dots and are classed as "b" the beaded and "g" the granular. This loosing of the bacilli like appearance is called "moniliform"—like a string of beads.

On account of the capsule and the large amount of fat this organism contains, it stains with difficulty. Fuchsin is a powerful stain, and phenol as a mordant, is used to in-

crease its penetrativeness; but even with this stain, the penetration of the stain into the organism is so slow that it must be hastened by heat. Once stained, it is almost impossible to decolorize, or take the stain away by using alcohol and HCl—hence the name given to this class of organisms, "acid fast." This decolorizing with acid and alcohol removes the fuchsin stain from all other organisms, except this "acid fast" group, which includes the Bacillus tuberculosis, the only acid fast bacillus found in the mouth. The Bacillus leprae, the cause of leprosy; the Smegma bacillus (gives up most of its stain) which is found in urine, and several others, one of which is found in distilled water, one in dung and one in butter and cheese.

The bacillus grows with difficulty on ordinary nutrient broth to which has been added 5% glycerin, but is grown easily on sub-cultures. A number of excellent media have been developed, as Petroff's medium—veal broth, to which has been added 10% glycerin; then 1 part of this mixture is added to 2 parts of egg, and 1 part of a 1 in 10,000 alcoholic solution of gentian violet, inoculate with the suspected sputum to which has been added an equal part of a 3% NaOH solution; incubate 30 minutes; neutralize with HCl, using litmus as an indicator, then centrifuge and stain and examine.

It also grows on dog, or horse serum, glycerin agar or broth. The growths appear in about 10 days at 37 degrees C., as minute barely visible grains, dull, wrinkled, chalk colored and irregular in outline.

The Bovine Type, or Mammalian Type. Stain more solidly and are shorter, straighter and thicker than the human type. They are much more virulent for rabbits and cattle. Fowls are little affected with this type. This type produces much of the miliary and intestinal consumption in man, while the human type produces nearly all of the pulmonary and laryngeal consumption in man.

The Avian Type, or Fowl Type. The cause of one of the common barnyard diseases. The organism resembles in appearance the human type but is more branching, and much more easily grown. This type can infect man and the lower animals. It is common to birds and chickens, appearing as yellowish-white nodules in the intestines and liver; the nodules vary in size from that of a pea to the size of a walnut. The organism is club shaped and more easily cultivated than that of the human type and also differs by a moist mucus-like growth; while that of the human type is brittle, warty

and dry. The avian type grows at the higher temperature of 40-50 degrees C. This type is less pathogenic to guinea pigs, but more so for rabbits.

The Cold-Blooded Animal Type. The organism affecting fish, frogs, et al., is longer, thinner and more contorted than the human type.

It is thought that these types can by passage through the different animals change from one to another type. They are all acid fast.

The human type can infect animals, and the bovine type can infect man.

The human type is very resistant to dessication; for the bacilli are viable in dried sputum in direct sunlight for a short time; in a darkened room they may retain their virulence for six or more months. They withstand 100 degrees C., for one hour, but when in liquids and heated, they are destroyed in 30 minutes at 60 degrees C.—Killed by boiling, as in milk, for 5 minutes. 5% phenol solutions, when mixed with an equal part of sputum, require 24 hours or longer. They will live in water for over a year and in ice or frozen products indefinitely.

Sputa expectorated on the roadway under the usual conditions even when exposed to the sun in summer for from two to fifty-two hours, that is, during nine successive days, induced the development of tuberculosis when guinea-pigs were inoculated from them. All the inoculations gave positive results, in one instance after eleven days of isolation. When the sputa had been deposited on snow beaten down in the driveway, the inoculations were all negative except in one instance in which the isolation had been only for thirteen hours. The sterilizing action of the sunshine is thus practically negligible for clumps of sputum such as the tuberculous expectorate.

THE TUBERCULINS

Original Old Tuberculin or T. O. Cultures are made in wide mouth flasks with veal glycerin broth; incubated two weeks until a pellicls forms, which sinks in about 4 weeks; filtered and evaporated on a water bath to 1-10 its volume and stored in a cool dark place, where it retains its virulence for months. The active part of this evaporated filtrate is an albuminous derivative, probably a nucleoprotein, which is insoluble in alcohol. This is used in testing cattle for tuberculosis. 0.25 to 2 mils according to the weight of the animal is injected. Before the injection the temperature is taken every 2 hours for one to two days. If a rise of 3

degrees C., in temperature is noted after the injection it indicates the cow is tubercular. No rise takes place in the healthy animal.

New Tuberculin. Evaporated in vacuo instead of ordinary heat.

Tuberculin Residium, or T. R. The ground living bacilli dried in vacuum, ground in salt water, filtered, centrifuged, preserving the supernatant liquid. The bacillus acts on tissues only.

Tuberculin Ober, or T. B. The clear liquid filtered from the original old tuberculin, containing the tuberculin, and is a 50% glycerin extract of the soluble products.

Bacillary Emulsion, or B. E. Suspensions of pulverized bacilli in 20% glycerin. It contains both the T. R. and T. O.

Antituberculin. 40% glycerin added to virulent cultures, sterilized 1 hour, filtered and 8 volumes of water added, then sterilized for 90 hours, the evaporated water being renewed every 12 hours.

Deny's Filtrate, or B. F.—Broth filtrate.

Bovaccine, or T. C., or Tuberculase. The bacilli with 20% glycerin.

T. R. and B. E. are given in doses of 1-4000 mgm. They are marketed in 1 mil bottles and are to be diluted with water and glycerin, 10 mills to 1.10 mil of the tuberculins. Treatment is begun by giving a very small dose and gradually increasing the dose of the injections.

Sensitized tuberculin is made by growing the bacillus in immune serum.

Non-virulent T. B. are living organisms, which, by their passages through animals or culture media, have had their disease-producing powers so weakened that they are unable to produce the disease. It is a question whether the bacilli when injected into the body will not become transformed into virulent types.

Tuberculins and the derivative from the bacillus have not been successful in the cure and diagnosis (except in cattle) of consumption, and they are becoming less used each year.

T. O., or Tuberculin Old, is used in the Calmette Eye Test which consists of dropping a drop of a ½ or 1% purified tuberculin into the eye. Redness in 12 to 24 hours is said to be positive.

The Cutaneous, or Von Pirquet Test. Consists of vaccinating the patient with tuberculin, which if positive will give the characteristic reaction.

The Moro Test. Equal parts of tuberculin and lanolin are mixed and rubbed on a place where the skin is thin, as the abdomen. If the reaction is positive, pustules appear, varying in size according to the extent of the disease, in 24 hours.

No test so far has proven reliable. The best test is the physical diagnosis confirmed by growing and isolating the bacilli and inoculating them into guinea pigs, and from the guinea pigs, reculturing.

Plaster Test. The skin of the forearm is cleansed with ether, a drop of tuberculin is placed on the ether-cleansed skin, and is immediately covered with a 3 cm. square of adhesive plaster, the edges being firmly pressed to assure sticking. In 24 to 48 hours, if eruption takes place under the plaster, the stage of the disease is said to be mild; but if intense red papillae vesicles and nodules appear the case is said to be advanced.

Albumen Test. 10 mils of the sputum are mixed with 10 mils of water; then 1 mil glacial acetic acid is added to precipitate the mucin. Filter and heat; or the nitric acid test is used for albumen.

Glycogen Test. The sputum is made alkaline with sodium carbonate, heated 1 hour so that the glycerin may be dissolved out, filtered and evaporated, then Tr. Iodine is added; and if a mahogany colored ring is seen at the juncture of the two liquids, it indicates the presence of glycogen.

Salicylic Acid Test. The patient is given 30 grams of salicylic acid, and the sputum is collected for the next 12 hours. Salicylate tests are applied, as the ferric chloride test which gives a violet color. It shows that an inflamed condition of the lungs exists.

Urochromogen Reaction. Urine is added to water, so that in a series of test tubes there will be 1-3 urine to 2 parts water in one tube, in another 1-6 urine and 2 parts water, in another 1-10 urine, in another 1-15 part urine to 2 parts water. The column of liquid in each test tube must be 3.5 cm. deep. The tubes are held over a white background and 0.1 mil of a 1 in 1,000 potassium permanganate solution is added. Looking down through the liquid, from the top, an increase of yellow at 15, 30, 60 and 120 seconds indicate a positive reaction. Deep yellow means that a higher dilution of urine is necessary. Pinkish or pink foam is suspicious.

The Precipitin Test. An emulsion of equal parts sputum and salt solution is boiled, filtered and placed in con-

tact with immune serum obtained from an ass immunized first with dead and then with living bacilli. Fornero uses 2 mils sputum and 4 mils of chloroform and incubates for 3 hours. The chloroform is decanted and 4 parts of saline solution is added to the residue, shaken and boiled for 2 minutes; filtered and placed by means of a pipette into test tubes half filled with saline solution. The reaction occurs in 15 to 20 minutes.

Complement Fixation Test. This test is due to the fact that antibodies appear in the blood after injections of tuberculine. Several strains of the bacillus are grown in alkaline broth to which has been added 1 gram of egg white and yolk, to each 250 mils of broth. When the growth has well developed, 95% alcohol is added, the whole shaken for 12 hours and incubated for 24 hours. It is then shaken six hours and filtered through a fine filter. The filtrates of the different strains are mixed and titrated for the anticomplmentary, hemolytic and antigenic properties. The antigen must be kept on ice. Human serum is used and the same technique is applied as in the Wasserman reaction. The tubes are examined in 1 and 2 hours after the last incubation in order to avoid confusing late laking.

Tellurite viability Test. Cultures on sterile concave slides are made, then 1 drop of 0.2% sterile solution of sodium tellurite is added, the covered glass placed over the culture and the slide incubated from 30 minutes to 2 hours. Black colors are seen if the bacilli are viable.

There are three methods in common use for the examination of sputum for the bacilli. In the antiformin method equal parts of sputum and 30% antiformin are placed in centrifuge tubes, permitted to stand for 12 hours, then centrifuged for 15 minutes. The Autoclave method, is placing the sputum in the original bottles, in the autoclave for 15 minutes at 15 pounds pressure—this process coagulates mucus and serous material and renders the tubercle bacilli innocous. The third method is the direct smear or filming of the sputum on the slide. The slides are stained by the ordinary carbol-fuchsin method. Some authorities use a saturated alcoholic solution of picric acid for a counter stain, claiming that the preparation is stained a soft yellow, easily penetrated by the light, thus making focusing easy and improving the chances of finding the acid fast organisms.

Many microscopists use the Gaffky-Brown scheme for tabulating the average number of bacteria per field in accordance with the following: 1. Only 1 to 4 in a whole preparation. 2. Only one bacillus on an average in many fields

Only one bacillus on an average to each field. 4. 2 to 3 acilli on an average to each field. 5. 4 to 6 bacilli on an verage to each field. 6. 7 to 12 bacilli on an average to ach field. 7. 13 to 25 bacilli on an average to each field. About 50 bacilli on an average to each field. 9. 100 or ore bacilli on an average to each field. 10. Enormous umbers on an average to each field.

The Zinc Precipitation Test of Ditthorn and Shultz for he Examination of Tuberculous Sputum—Render the sputum omogeneous, then place equal amounts in two sedementing ubes, to one tube add 0.5 mils of a 20% ferric chloride so- tion and to the other tube add 0.5 mils of a 20% solution f zinc acetate or zinc chloride, set the tubes aside for sev- al hours and when the sediment has been precipitated, pour ff the supernatant fluid and transfer the remained of the uid and sediment to a square of filter paper, several layers hick. The filter papers absorb the fluid and while the sedi- ent is still damp, spread on slides in a moderately thick yer. Air dry and stain as usual.

EXERCISE

1. Select a yellowish spot from a shallow dish lined with lack paper on which the sputum has been poured.

2. Spread evenly over a slide by means of another lide, crushing the tubercles.

3. Air dry.

5. Flame slide three times.

6. Stain in hot carbol-fuchsin solution (STEAMING, OT BOILING) for 5 minutes. Do not permit the slide to ecome dry, but replace the evaporated stain with more tain. Keep the slide covered with stain. Slides may be tained cold by immersing in the solution for 10 or more ours.

7. Wash with water.

8. Decolorize with 2% HCl in alcohol, UNTIL NO RED S SEEN UNDER THE LOW POWER.

9. Wash with water.

10. Counterstain with Loeffler's solution for 2 minutes.

11. Wash with water, dry and examine. If clusters of ed are seen, the specimen is not properly stained and must e made over. If no red is seen, make a permanent mount with canada balsam, and examine with the 1-12 inch lens. Remember that only the Bacillus tuberculosis is red, every- hing else must be blue.

12. Carry out the antiformin test as given under the formulae of stains.

Moro Sulphuric Acid Test. The slide is stained wit fuchsin 0.5 grams, absolute alcohol 100 mils, phenol 2.5 mi and water 100 mils, (this solution must stand 24 hours b fore being used) for 20 minutes, the slide is washed wit water, then decolorized in 1% sulphuric acid for 15 minute washed with water and counterstained with a 1 in 4,0 methylene blue solution for 15 minutes, washed with wat and examined.

Gabbot's Method. Stain with carbol-fuchsin by stea ing for 6 minutes, wash in water until no more stain com away; then the slide is flooded with Loeffler's stain for seconds, again washed with water; then immersed in 95 alcohol for 24 hours and examined. Acid fast organisr retain the red stain.

SPUTUM FROM CONSUMPTIVE.

There are at least 4 distinct types of pneumococcus: riedlander's bacillus, Pfeiffer bacillus, and various types of reptococcus of which the hemolyticus is the most virulent, oncerned in the production of the various kinds of pneuonias. The pneumonia caused by the plague bacillus is he most fatal of all, practically causing 100% mortality. is thought that any bacteria capable of growing and riving in the lung tissue may cause pneumonia.

As the Pfeiffer bacillus will thrive only on mediums conaining hemoglobin, it is probable that it is associated with he influenza pneumonia, which is of the hemorrhagic type. ome types of streptococcus are always found in lobar pneuonia, so that it is probable that different types of bacteria elects some special lung tissue to live and thrive upon and et practically every case is one of mixed infection.

The pneumonias are characterized by anemia, a reduction of hemoglobin to 70% or less. A leukocytosis in proportion to the area of tissues involved, Polymorphoneuclears, olynuclears, and neutrophiles predominate but are lessened n convalescence. In children there is a lymphocytosis. Blood causing the rusty brown sputum and a heavy urate sediment in urine on standing is also characteristic.

The diplococcus pneumoniae or the pneumococcus is a arge diplococcus, slightly elongated and consisting of two anceolate shaped cocci with usually the points together and sometimes growing singly or in chains, (surrounded with a capsule appearing as a hyaline zone, when found in animal exudates). It stains a blue-black with the Gram stain and is surrounded by a hyaline zone. It grows on meat broth made 0.3% acid to phenolthalein to which has been added 4 drops of rabbit blood serum to each 5 mils of broth and grows on solid media as small moist translucent, granular well defined edged colonies—Acidifying and usually coagulating milk and fermenting inulin (difference from the associated streptococcus). Dried cultures remain viable for several weeks, and in vacuum dried spleens of infected mice, which have been bottled, sealed and kept cold, they remain viable for months.

There are 4 distinct types of pneumococcus. Types 1, 2 and 3 are found in severe cases in man and give constant immunity reactions and are the cause of 80% of lobar pneumonia. The type 4, is composed of a number of strains differing in seriological reactions and are rarely found in fatal pneumonias, but are found in normal mouths.

The types 1 and 2 cause most of the pneumonia case and are rarely found in normal mouths. Type 1 cause 33.3% of pneumonia cases and a mortality of 25%; type causes 33.5% of cases and a mortality of 32%; type causes 13% of cases with a mortality of 45%; and type causes 20.3% of the cases with a mortality of 16%. Typ 3 is the most virulent and type 4 the least virulent. Patho genicity may be increased in any of the types by passag through animals, or transfer from person to person, an through cultivation on different mediums.

No soluble toxin has been discovered; agglutination ma be accomplished in many cases, but not over 50 positive.

An immune serum has been used for type 1 infection by injecting intraspinally 20 mils, and subcutaneously 5 mi and repeated every 5 hours until from 80 to 100 mils hav been injected. Vaccines have been used for types 1, 2 and More success has been obtained by using the lipo-vaccine which are suspensions of the dead bacteria in cotton seed oi giving one dose only of 10,000 dead bacteria. By using th oil instead of saline, the bacteria are more slowly absorbe and the negative phase and lowered resistance is avoide Pooled serum from convalescents has been used and has re duced mortality to some extent.

ISOLATION is accomplished by subculturing, or b mouse inoculation. Small particles of sputum are washed times with sterile saline solution and powdered in a steri mortar, and from 0.5 to 1 mil is injetced intraperitoneall Mouth organisms will not thrive but Bacillus influenzae, M crococcus catarrhalis, staphylococcus and streptococcus wil

From 5 to 24 hours (average 7 hours) after inoculatio the mice become sick and at this time aspirate by means c the hypodermic syringe a drop of liquid from the inoculatio sac, stain and examine for the pneumococcus. If there ar many pneumococcus in the injected material the mouse wi die; if only a few are present the mouse will not die an must not be killed until the test shows pneumococcus. The kill the mouse and determine type.

AUTOPSY. Make streak cultures from the peritone cavity, heart blood and injection sac (if any) and from centr fugings of mixed heart blood and peritoneal cavity fluid o blood agar medium; slides are made, stained and examine

DETERMINING THE TYPE. 0.5 mil of a pure 18 hou old culture or the centrifuged washings of the peritone cavity are mixed with 0.5 mils of serum of the various type 5 different tubes with the cultures or washings must be use

with each test for each serum; the serum is to be of various dilutions ranging from 1 to 20. After being mixed the tubes are incubated on the water-bath at 37 degrees C., for 1 hour and the agglutinations noted. The immune serum which is agglutinated indicates the type of organism.

PRECIPITATION TEST. Peritoneal washings, centrifuged and supernatant fluid only used, is mixed with equal parts of the different immune serums of various dilutions ranging from 1 to 20. The tubes are permitted to stand at room temperatures for 1 hour and then examined. The tube which shows a precipitation indicates the tube of organisms which are the same as the type of serum contained in the tube.

IDENTIFICATION OF THE MENINGOCOCCI

5% unheated clear sterile horse serum is added to a 1% glucose broth made from veal, and acid from 0.5 to 0.7 with phenolphthalein. Place 1 mil in each of a series of small tubes. Fish out suspected colonies and seed in the tubes; incubate at least 12 hours, when the typical meningococcus colonies can be seen.

On plate cultures the following organisms may cause complications: Micrococcus flavus, crassus, pharyngis siccus and catarrhalis; Bacillus infuenzae and an unclassified Gram positive bacillus; and especially in the hands of a beginner, staphylo and streptococcus. Due to the presence of the horse serum a normal horse serum control is present, hence Micrococcus flavus, crassus and pharyngis siccus and the unclassified Gram positive bacillus will show firm agglutination. The baccili culture may show a slight turbidity over the agglutinated sediment; the diplococci cultures show a clear supernatant fluid over the agglutinated sediment. Due to the absence of hemoglobin the Bacillus influenzae will not grow in this medium but the Micrococcus catarrhalis grows with a dense turbidity and often with a pellicle.

The Gram positive organisms are: Staphylococcus—growing with a dense turbidity, agglutinated masses in the sediment and often with a pellicle. Streptococcus—growing with a clear or turbid supernatant fluid and an agglutinated sediment. Meningococcus—growing with a slight turbidity and a slight sediment and the sediment emulsifying uniformly upon shaking.

To the tubes showing the meningococcus characteristics add 0.1 mil of a 1 in 10 dilution in normal saline solution of a high titer polyvalent antimeningococcus serum to each tube. Incubate for 2 hours on the water bath and examine.

The tubes containing the meningococci exhibit distinct agglutination, the organisms in the tubes are verified by staining with the Gram stain. The tubes containing other organisms than the meningococcus remain unchanged. All tubes are regarded positive which show agglutinating masses of Gram negative diplococci of typical meningococcus morphology.

Sheep serum agar medium, or veal infusion agar medium with 2% dextrose and 0.4 positive to phenolphthalein, or sheep serum 1 part and 3 parts water, sterilized 40 minutes at 15 lbs. Then 1 part is added to 5 parts of plain nutrient agar medium and incubated 72 hours as a sterility test, or defibrinated human or rabbit blood in glucose agar medium sufficient to give it a pink color and tested for sterility at room temperatures for 36 hours.

For stock cultures inoculate slants and incubate not over 16 hours, then keep at room temperatures (not in ice box) for 36 hours; then transplant to litmus serum agar medium and the meningococcus will show as pink colonies. Micrococcus catarrhalis will be bluish or gray and Micrococcus flavus, opaque and yellowish-green.

Material taken by swabs from the postpharyngeal space is to be isolated as given above and tested with agglutinating serum.

Agglutination Test. The slant cultures are washed with 2 mils normal sterile saline solution and 0.2 mil is used for each dilution of serum (polyvalent anti-meningococcus horse serum). The dilutions should be 1 in 50, 1 in 100, 1 in 500, 1 in 1,000 and 1 in 2,000, and 0.8 mil of the diluted serum is placed in each tube with the 0.2 mil of culture. The tubes are incubated 16 hours at 55 degrees C. The meningococcus should agglutinate in dilutions of 1 in 200 or more, although some strains will not agglutinate in dilutions over 1 to 100 Micrococcus flavus will agglutinate normal horse serum. For determining the types of meningococcus, monovalent anti-meningococcus serum must be used; this is obtained from immunized rabbits, immunized to one type or strain.

Success is obtained by growing the meningococcus by the partial tension method, which consists of placing a slant culture on human serum glucose agar medium in one tube and a culture of Bacillus subtilis in another tube; the 2 tubes are fastened together by means of a rubber hose placed over the open ends of the tubes. The B. subtilis reduces the oxygen supply.

When meningococcus is seeded in a petri dish, the dish must first be warmed and kept warm until placed in the in-

ubator. The medium must always be moist and the dish hould be inverted when placed in the incubator. When rown on whole blood mediums it shows no green color but ppears as moist, elevated colonies, ill-defined outline, and paque with a faint smoky-gray-bluish tint.

Yeast medium for prolonging the viability of meningoccus. 10 grams of yeast are macerated in 100 mils of ater for 20 minutes, then steamed for 2 hours at a temperture of not over 100 degrees C., clarified by adding dialyzd iron and filtering through glass wool. To 1% agar conaining 2% peptones and 0.4% potassium phosphate add an qual amount of the prepared yeast and adjust to 7.6 to henolphthalein, and place 10 mils in each test tube; sterilze for 30 minutes at 15 lbs. Inoculate by making stab culures. The tubes are sealed with paraffin or sealing wax nd placed in the incubator until the culture is needed. Culures will remain viable for a number of weeks by this method.

TRENCH FEVER

In the spring of 1915, there began to appear in th British army in France a large number of cases of acut infections resembling influenza, and on account of the case being among the men in the front line it was called 'trenc fever.'

It is characterized by a sudden onset of fever, dizziness headache, back and leg ache which later becomes localize in the shins. The temperature is high for three or fou days, then becomes lower with a rise after an interval of on to two days; the defervescence takes place about the eight day and followed at regular intervals by relapses. There ha been no mortality. It has occurred among the men who ha recently been in the front line trenches and the personnel o the hospitals in which trench fever patients had been care for.

Trench fever is caused by an infectious organism, no definitely isolated, but which is said to closely resemble th organism, or spirochete, causing rat bite fever and probabl is a protozoon which hitherto has been found in lizzards, rat and dogs, but not in man.

The transmission is by contagion, by louse bite, or b the excreta of lice being rubbed into the skin by scratchin the bite. The incubation is from five to thirty-five days.

The prevention is like that of typhus fever, isolation o the patient and the men and keeping the patients free fro lice.

INFLUENZA

The term influenza was adopted by the College of Physicians in London in 1782; yet the disease was known to Hippocrates and other ancient physicians; and there is a record of an epidemic occurring in 1173 and frequently from that time until the present time. An epidemic swept the whole world near the end of the eighteenth century, and reached in 1781, Siberia, Russia, China and India in the autumn and in December it invaded successively Finland, Germany, Denmark, Sweden, England, Scotland and the Netherlands, France, Italy and Spain. It was spread by communication with caravans and the sea. It was called "febris catarrhalis epidemica beigna," by the Russians in 1782.

It occurred as an epidemic in the United States in 1833, 1847, 1855, 1889 and 1890, and in 1914 it caused 136,000 deaths; and again in 1918 and 1919, when it caused over 600,000 deaths and an economic loss of over $4,000,000,000. It caused more than five times the deaths of the World War. Congress was asked to appropriate $1,500,000 for investigation and fighting the disease. It attacked those from 20 to 30 years of age more frequently, while the epidemic of 1890 attacked one million persons over the age of 30. Over one-half of the population had the disease in the last epidemic and as it was somewhat world-wide it is estimated that over six million deaths were caused by the influenza. In the Civil War, during 1862 and 1863 there were 85,677 deaths of catarrh (influenza) out of 134,397 cases.

Cromwell died in 1656 of "ague" and England was one vast hospital from influenza in 1712 it was called "La Grippe" and the Americans shortened this to "Grip."

It appeared in India as an epidemic which assumed the proportions of a national calamity; and in British India it is estimated that during 1917 to 1919 it caused over 6,000,000 deaths, the people sickening and dying so fast that the hospitals were choked with patients, and the streets and lanes were full of dead and dying people.

This epidemic was first observed in Vienna in 1916 and 1917. It spread from there to all parts of the world; but it is probable that it was caused by a continuation of the 1890 European epidemic; and spread to Austria, England and France and from there to the United States.

The infectious agent is probably some virulent strain of the Bacillus influenzae or Pfeiffer's bacillus. This bacillus is frequently found in throats of patients with measles, per-

tussis, diphtheria and scarlatina, more especially if these are complicated with some form of purulent bronchitis, or broncho-pneumonia. The bacillus is probably only one of the causative factors; the others may be hemolytic streptococcus, pneumococcus of the various types, micrococcus catarrhalis, non-hemolytic streptococcus and others. There have been cultured from influenza patients, one or all being present at the same time. The influenza bacillus produces a filterable toxin which is fatal to rabbits in small doses, and the toxin is produced rapidly even in cultures 6 to 8 hours old. The toxin is destroyed rapidly unless placed on ice. No single causative organism has been discovered, but the disease is due to some unkown virus—which causes inflammatory changes.

It is probable that the poison of B. influenzae contains two poisons; the first, the more important one, a true soluble toxin, filtrable, thermolabile, against which antitoxins can be produced; the second, present also in the vaccine of B. influenzae, also filtrable, but differing from the first poison in its thermostability, and in fact that it is not detoxicated by the antitoxin.

B. influenzae produces a filterable poison lethal for rabbits when given intravenously. It is only partly destroyed when heated to 55 degrees or when boiled. Rabbits can be immunized to at least 4 or 5 m. l. d. of this poison and the serum neutralizes the poison in vitro. Influenza bacterial exts, are poisonous to rabbits in relatively large amounts. The Berkefeld filtrate of the bacterial exts, is nearly as toxic as the exts. themselves. Boiling does not destroy the toxicity, nor it it reduced in vitro by the immune serum.

The source of infection is the secretions from the nose, throat and respiratory passages, or through carriers.

The incubation period is from 1 to 4 days, generally 2 days.

It is transmitted by direct contact, or indirect contact through the use of handkerchiefs, towels, eating utensils, et al., contaminated with fresh secretions. Droplet infection plays an important part in the transmission.

The period of communicability is as long as the person harbors the causative organisms in the respiratory tract.

The methods of control are by quarantine of the infected person and the proper precautions taken of his environment.

Recognition of the disease is by the characteristic clinical manifestations, and bacteriological findings. The symptoms are sudden pain, headache, catarrhal conjunctivitis,

sore throat, stupid and dazed condition, drowsiness, eye pain, blurred vision, no rapidity of the pulse or leukocytosis, joint pains, prostration, a temperature rarely exceeding 104 degrees F. Mucus flows from the nose and mouth, sneezing, hoarseness, and unless proper care is taken pneumonia is likely to set in.

It may be followed by extreme weakness and sometimes by epidemic lethargic encephalis, the symptoms of which are high fever, drowsiness and paralysis of the cranial nerves, especially the ocular nerve—the patient sometimes going into a profound sleep resulting in death. Delirium is seen in some cases. The prognosis is good; in 168 cases there were 37 deaths. The stupor varies from 2 days to 5 weeks and in some cases the symptoms persist for months.

Little medicine is needed in influenza. Acetysalicylic acid every 1 to 3 hours, or acetphenetidin 0.1. Warm antiseptic baths twice daily, mouth and nose cleansing, proper elimination from the kidneys and bowels.

Isolation. Bed isolation of infected patients during the course of the disease. Screens must be placed between beds in wards or rooms where there are two or more patients.

Immunization. Bacterial vaccines have been used with only partial success. The toxemia of influenza is neutralized to some extent by the serum obtained from convalescent patient's blood. As yet there is no known immunization or immunity. In some localities there were 3 or 4 returns of the epidemic at intervals of about 12 weeks. In several cases the same patient had the disease 3 different times.

The bacterial vaccine generally used during the epidemic of 1918 and 1919 was composed of:

Pneumococci, Type 1, 10%; Type 2, 14%; Type 3, 6%;
Typye 4 ..... 30%
Pneumococci and the allied green producing diplostreptococci 30%
Hemolytic streptococci 20%
Staphylococcus aureus 10%
Influenza bacillus 10%
Given in doses of 5 millions.

The official army formula.—30 m" B. influenza, 100 m" pneumococci, 40 m" streptococci in 9 minims salt solution. Dosage used was at first m iii. (and later, for first dose, m. v., and m. viii for the second).

Other bacterins were tried, consisting of various mixtures of bacteria, but with little success.

Quarantine. A strict quarantine should be enforced and continued for 2 weeks after the patient leaves the bed, and before quarantine is released there should be 2 negative findings at intervals of 3 days.

Concurrent disinfection. All discharges from the patient as the nose, mouth and other respiratory passages should be thoroughly disinfected or burned.

Terminal disinfectant. Thorough disinfection with formaldehyde, or other disinfection. Thorough cleaning, airing and sunning.

General measures. The attendant should wear a gauze mask. During epidemics all crowded assemblages, street cars, et al., should be avoided. Education in regard to the danger of coughing and spitting. Patients must be kept in well ventilated, warm rooms on account of the tendency to develop broncho-pneumonia.

The Surgean General recommends the following rules as preventative measures:

1. Avoid needless crowding—influenza is a crowd disease.
2. Smother your coughs and sneezes—others do not want the germs you would throw away.
3. Your nose, not your mouth, was made to breathe through—get the habit.
4. Remember the three C's—a clean mouth, clean skin, and clean clothes.
5. Try to keep cool when you walk and warm when you ride and sleep.
6. Open the windows—always at home at night; at the office when practicable.
7. Your fate may be in your own hands—wash your hands before eating.
8. Don't let the waste products of digestion accumulate—drink a glass or two of water on getting up.
9. Don't use a napkin, towel, spoon, fork, glass or cup which has been used by another person and not washed.
10. Avoid tight clothes, tight shoes, tight gloves—seek to make nature your ally, not your prisoner.
11. When the air is pure breathe all of it you can—breathe deeply.

The Secola of Rome states there were in Italy 800,000 deaths during 1918 caused by grip, or about 60% more than the total deaths caused by the war. The same paper estimates the deaths by grip throughout the world were double the deaths caused by the war.

Keep Well Series No. 4, U. S. P. H., states: The person attending the patient should wear a double layer of gauze or other thin cloth across the mouth and nose as a face mask whenever near the patient so as to prevent the droplets containing the germs coming from the patient's mouth from entering and lodging on the mucosa of the mouth and throat of the attendant. Even though you may not contract the disease if the bacteria lodge in your throat, they may grow there and you may act as a carrier and thus spread the disease to others.

The influenza bacillus grows well on 94 mils nutrient agar, to which 5 mils of a 2% sodium oleate solution and 1 mil of sterile defibrinated rabbit or human red blood cells is added (blood serum inhibits the action of sodium oleate). The red blood cells are added to the medium just before using. Broth is made in the same way and in the same proportions. The cultures are incubated for 48 hours; the Gram negative cocci, Micrococcus catarrhalis, staphylacoccus and diphtheria bacillus will grow on this medium, but penumococcus, hemolytic streptococcus and Streptococcus viridans will not grow.

Influenza bacillus may be readily isolated by mixing equal parts of sputum or bronchial secretions with a 0.5% sodium taurocholate solution and setting aside for 30 minutes. In this time nearly all other micro-organisms will be killed, such as the pneumococci, staphylococci, micrococcus catarrhalis, etc. At the end of 30 minutes streak cultures on human blood agar plates are made, incubated at 37 degrees C. The influenza bacillus grows as minute colorless, transparent drop-like colonies.

B. influenza grows with a rapid production of toxin in the following prepared medium. Whole blood added to agar medium, then boiled for 1 minute and sloped. The coloring matter of the blood and the proteins are precipitated and do not intefere with the growth. Or blood corpuscles may be substituted for whole blood, or 1 mil of blood boiled in 10 mils of water and the clear portion added to nutrient agar medium, or an equal volume of normal sulphuric acid to blood; or the same effect may be obtained by using normal caustic soda solution, instead of the acid. This will cause a breaking down of the hemoglobin into a brown precipitate and a brownish colored liquid; this liquid when added to nutrient agar medium and inoculated with B. influenzae will cause profuse growths for making bacterial vaccines. By

the addition of brilliant green to this medium pneumococcu staphylococcus and streptococcus are inhibited. Cultures the B. influenzae may be kept viable for a long time on t blood agar medium.

The Bipolar Bacillus of Influenza Septicemia is found conjunction with the B. influenzae, and is rod-shaped tendi to an oval form, Gram negative, with distinct bipolar stai ing and a tendency to morphological variations, non-motil and grows best on plain agar medium at 37 degrees C., a is aerobic. It will not produce gas in glucose broth nor ac in glucose, lactose, or saccharose, and will not liquefy ge atin, or coagulate, or render litmus milk acid. It probab is one of the hemorrhagic septicemia group and differs fro this group by its lack of virulence; for injections of sma doses into rabbits, guinea pigs and rats will not produ death.

THE HEMOLYTIC STREPTOCOCCI

This term is given to those streptococci which on pla blood agar media cause a wide, clear, complete zone of hem lysis about a small, central gray colony. This zone is usua ly 2 to 4 mm. across.

Broth cultures, when added to a suspension of red blo cells, will hemolyze them. The common streptococci in d lute blood media after several days may cause colored incomplete hemolysis—the differentiation test. The strept cocci which cause hemolysis differ in many other ways.

There is an acquired immunity to the hemolytic strept coccus. The infection causes a rise in temperature and leukocytosis. These organisms are very pathogenic to t horse and cow, affecting the respiratory centers, and also i the cow causing sore udders, hence many sore throat ep demics may be traced to this source.

Many diseases are attributed to these organisms, erysipelas, pneumonias, influenza, sore throat, endocarditi some of the contagious fevers, and they are constant measles.

Their virulence is very great, for if 0.5 mil or less of broth culture is injected into a 1,000 gram rabbit it will ki it in a short time.

The dead organisms when injected into man, cause formation of antibodies, opsonins and sometimes agglutinin

Their hemolytic activity is caused by a hemolysin call streptolysin.

In 1903 Schottmuller classified these organisms, discar ing the old term, Streptococcus pyogenes, and used the term

S. viridans, S. hemolyticus and S. mucosus, on account of their reaction and appearance on blood media. Horder and Andrews classified two of their varieties as S. pyogenes and S. anginosus which were hemolytic.

Microscopically, these streptococci are spherical or slightly oval and form definite chains (sometimes very long chains), and if seen in the tissues multiplying rapidly they appear as in pairs or short chains. Pairing is often noticed in the chains but not as frequently as seen in Pneumococci or S. viridens.

ISOLATION AND IDENTIFICATION OF THE HEMOLYTIC STREPTOCOCCI

The material for culture is taken on an ordinary sterile diphtheria swab from the throat. If there is much pus, pleural exudate or sputum, these may be collected in sterile bottles or tubes without antiseptics and kept cold until cultured.

For culturing, swabs are moistened with a little sterile salt solution if necessary; sputum is washed with sterile salt solution and a kernel is selected.

Blood agar medium in tubes, or petri dishes, is inoculated, or streaked on the surface of the medium near the edge with the swab; then with the platinum needle the surface is streaked. In this way a greater dilution at points farthest from the spot touched by the swab is secured. By cutting the surface with the needle as it is drawn away from the inoculated spot an opportunity is given for deep growth. Incubate 18 to 24 hours at 37 degrees C., keeping the incubator atmosphere humid. Seek well defined colorless zones of hemolysis; these zones should show no greenish or brownish color under the low power of the microscope.

Transfer a single typical colony to a tube of nutrient broth and incubate over night or until a good growth is secured; stain some of the organisms by the Gram method and study the length of the chains, the side and the appearance of the cocci in the chains.

Mix 0.5 mils of the broth culture with 0.5 mils of a 5% suspension of washed rabbit blood corpuscles in a physiological salt solution. Incubate 2 hours at 37 degrees C., the blood will be laked by the hemolytic streptococci.

To 1 mil of the bouillon culture add 1-5 its volume of sterile ox bile. Observant room temperature for 1 hour. Under certain conditions pneumococci may cause a hemoly-

sis on blood agar. Solubility in bile distinguishes them from the hemolytic streptococci.

All strains of hemolytic streptococci grow poorly in plain broth, but grow fairly well in glucose broth to which 10% blood has been added, with a uniform clouding, and a tendency in some strains to flocculate and sediment.

Additional tests may be made by replating the broth cultures in blood agar and studying the deep as well as the surface colonies. The sugar reactions are important. Hemolytic streptococci have a reaction with lactose, salicin and inulin, and but little or none with mannite.

Milk is coagulated in 7 days; if no coagulation occurs by this time, the tube of milk culture is immersed in boiling water for 10 minutes to determine whether coagulation will occur.

Inoculations of rabbits may be made intravenously with not more than 1 mil of the broth culture, or of mice intraabdominally with smaller amounts.

For the blood cultures horse blood is the best; but human and rabbit blood gives nearly the same results. A 9 cm petri dish should contain 12 to 15 mils of blood agar so as to form a layer about 3 mm. thick on the bottom of the dish. The medium must be kept cold before using; if laking or sedimentation has taken place in the medium, it must not be used. The medium must not be kept too long, or the surface will become dry, and these organisms will not grow unless the surface is moist. It is best to make the medium as needed.

DIAGNOSIS OF WHOOPING COUGH BY CULTURAL METHODS

As the Bacillus of Bordet and Gengou or Bacillus pertusis resembles that of B. influenzae, the diagnosis is somewhat difficult. The mucopurulent flocculi are fished out of the last portion of the expectorate, washed in sterile saline solution and then streaked across the surface of blood potato agar medium. After 4 days' incubation the colonies are typical, resembling tiny drops of polished and glistening mercury. The organisms are then tested with agglutinating serum from rabbits or horses immunized with the B. pertusis. In small children where no sputum is available, the children are made to cough against plates of the culture mediuus, held a few inches away from the mouth. After the attack has lasted for 4 weeks or longer cultures are hard to obtain. There should be at least 2 negative cultures before quarantine is lifted.

RAPID METHOD FOR THE IDENTIFICATION OF DYSENTERI BACILLI

Stools containing blood and mucus with little or no fecal matter are plated by picking out mucus shreds, washing with sterile saline solution and then streaking on modified Endo's medium which is a 1.5% agar beef or veal broth, titrated to 7.6 to phenolpthalein, then adding 1% lactose and 1% by volume of decolorized basic fuchsin indicator prepared by adding 1 mil of 10% basic fuchsin to 10 mils of 10% sodium bisulphite solution. The lactose and this indicator are added to the plates just before they are poured. Eosin-blue medium is also used; it is made by adding to veal broth agar medium titrated to 7.2 to phenolphthalein 1% lactose, 2 mils of a 2% solution of yellowish eosin and 2 mils of 0.5% solution of water-soluble methylene blue. Both types, Flexner and Shiga, are partly or wholly inhibited on alkaline mediums.

BACILLUS BOTULINUS

Is the cause of poisoning from spoiled canned animal or vegetable foods. The vegetable food poisoning occurs in cases where the food has not been sufficiently cooked before preserving.

The bacillus reproduces by spores. It is not known how they enter canned goods but they are not found under the skin of fruits. Hence only sound fruit or vegetables should be used for preserving. Bacillus botulinus in canned goods causes gas bubbles in the jars, the tops are sprung and the tops and bottoms of tin jars are blown and when opened a squirt of liquid is seen. There is an odor of rancid cheese and a mushy or disintegrated appearance of the solid contents of the jars.

The toxin in these jars may be destroyed by 5 minutes boiling which will not kill the spores. The toxin is only produced in material sealed in air-tight containers for a week or more; but as the bacillus produces no toxin in the human body, there is no danger from eating uncooked or freshly cooked unsealed vegetables. The slightest taste of the toxin is fatal, so that suspicious canned goods must not be tasted to see if they are spoiled.

Isolating the spores from foods. Inoculate 0.1 mil of the suspected food into tubes of melted 2% agar medium; the tubes are shaken and immediately cooled and sealed with paraffin, bees-wax, or zinc oxide. The tubes are incubated

at 28 degrees C. Or, use a medium made of sheep brains cooked in water and squeezed through cheese cloth, and sterilized in test tubes. The tubes are inoculated and covered with oil and incubated.

WATER

Water may be divided into: (a) Good water which by tests and examination of the watersheds has been found to be potable, free from pathogenic micro-organisms, excessive minerals, or other substances which would render it unfit for human use. (b) Polluted or contaminated water containing organic waste, vegetable or animal organic waste; and (c) Infected water—water containing pathogenic micro-organisms.

The sources of water supply are: (a) Rain or snow water, which when collected in the country is pure. It should be filtered before storing or using, so that all dust particles will be removed. It must not be stored in metallic tanks, as iron, lead, zinc and other metals will be attacked by it; (b) Surface water—comes from ponds, rivers and lakes. These are the natural sewers of the regions which they drain. No stream draining an inhabited region can be considered safe without some method of purification. Lakes and ponds are excellent water sources when they are kept free from pollution with human and industrial waste. These waters should be purified before using. Impounding reservoirs as artificial ponds or lakes should have the water purified before using; (c) Ground waters include wells and springs. This water in passing from the surface of the earth to the store houses as the wells and springs takes up a large amount of carbon dioxide which is set free from organic decompositions. This acidulated water becomes a solvent for lime and other mineral constituents, thus making the water harder than surface water. Ground waters exist as rivers or large bodies in lime-stone regions. Wells may be shallow or deep, dug or drilled. A shallow well is one which has been dug and lined with stone or brick. Deep wells are usually drilled and are 100 or more feet deep without passing an impervious stratum. These wells are wrongly called artesian wells. An artesian well is one which passes through an impervious startum into a pervious one beneath, in which the water rests upon another impervious stratum. All wells must be carefully constructed, so that no surface water may seep in, and cased at least 18 inches above ground to prevent frogs, in-

sects, etc., from getting in. Springs are natural wells outcropping where geological formations are favorable. If the overlying layer of soil is thin, the contaminations in the surface water may not be removed, and the water becomes polluted. They should be cased in stone or cement.

Water supplies are contaminated by vegetable and animal refuse, human and industrial wastes. Vegetable refuse and industrial wastes do not cause disease in man, but the animal refuse and human waste may cause diseases, as typhoid, consumption, etc. The liability of polluted water to cause disease depends upon the amount of pollution and the directness with which it reaches man. If the infectious material is large in amount but takes a long time to reach the consumer, the danger is less than when the amount is less and the time shorter.

Personal investigation of contaminated water supplies should include: The vegetable origin of contaminations; the animal and human origin of pollutions; the relative amounts of such pollutions; and the directness of such pollutions.

Sanitary Analysis of Water Consists of:

1. Physical examination to determine color, turbidity, odor and taste. Odors and tastes are objectionable. The odors develop more frequently in surface water and are caused by algae, diatoms, protozoa and other micro-organisms. In deep wells hydrogen sulphide and other inorganic compounds cause odors. When water is stored too long a time odors and tastes develop. Undecomposed organisms containing volatile oils also cause odors, as pig pen odor is caused by Amoeba; aromatic odors by asterionella and fishy odors by uroglena. These tastes and odors usually can be removed by aeration and copper sulphate treatment. The color is chiefly from dead leaves, and plant parts. Water containing iron is clear when leaving the ground but on oxidation by the air the ferrous salts change to insoluble ferric salts which cause the rusty yellow color. Color may be removed by bleaching, precipitation, oxidation or coagulation by adding iron sulphate, lime or aluminum sulphate. Turpidity or muddiness is due to clay or earth and is removed by a coagulant or by filtration.

Water may have an alkaline reaction due to the carbonates and bicarbonates of calcium and magnesium. Crusts on boilers are caused by sulphates and silicates. The acid constituents are aluminum and iron sulphate.

2. Microscopical Examination. This is to determine the presence of pathogenic bacteria and the organisms which

produce objectionable odors and tastes and also to determine the presence of micro-organisms of pollution which belong to the chlorophyceae, crustaceae, cyanophyceae, diatomaceae, fungi, protozoa, rotifera, etc. It is also for the purpose of determining sewage contamination, indicating the self-purification process of streams, the turpidity, identifying the source of the water, et al.

There are very few organisms found in lake water during winter. Limnetic organisms are those which grow and thrive in the open water. Littoral organisms are those which grow and thrive on the shore.

3. Chemical analysis to determine the total solids, the amount of organic matter, ammonia-nitrates, chlorine, mineral, et al.

BACTERIAL ANALYSIS OF WATER

The quantitative analysis is to determine the number of bacteria.

Qualitative analysis requires much time and skill for the cultivation, isolation and determination of the different kinds of micro-organisms. The principal value of qualitative analysis is the determination of sewage contamination. There should be less than 50 colonies of bacterial growth to the mil of water and it must be free from all liquefying bacteria, coli communis and others. The coli group is determined by the fermentation tube test. Bacillus typhosus is difficult to detect and unless several months are used in the isolation, one must depend on the presence of associated bacteria of the bowel, such as the coli group, which produces gas in the fermentation tubes. Bacillus vulgaricus and bacillus aerogenes also produce gas and usually are from contamination of animal bowel contents.

American Public Health Association Standard for Bottled Waters.

1. The total number of colonies developing on standard agar plates incubated for 24 hours at 37 degrees C., should not exceed 100 per mil. The estimate must be made from not less than 2 plates, showing such numbers and distribution of colonies as to indicate that the estimate is reliable and accurate.

2. Not more than one out of five 10 mil portions of any sample examined shall show by the method of the Public Health Service, the presence of organisms of the coli group; they shall be absent in 1 and 0.1 mil portions.

Samples of water for bacterial examination must be collected in sterile flasks or in the regular 100 mil glass

stoppered bottles, which have been treated as all glass ware used for bacteriological work—that is, carefully washed and rinsed and sterilized by heating for 1 hour at 165 degrees C. If it is not possible to culture the water immediately after taking the sample, and transportation is necessary, the bottle must be packed in ice and kept below 10 degrees C., for 1,000 colonies in the fresh sample, will develop into 100,000 or more in 6 hours, unless packed in ice.

Care must be taken to avoid contamination of the stopper or neck of the bottle by the fingers. Samples of tap water should be taken by permitting the water to flow for 5 minutes, then placing the bottle under the water flowing from the tap; the stopper is carefully removed so that the bottle may fill; it is then replaced, the bottle taken out of the water and unless cultures are to be made at once, packed in ice and a label or tag fastened to the bottle, with the time and place of sampling. Well, spring and river water should be taken at least 1 foot below the surface; or it is best to take samples half way between the top and bottom of the water. Bacteria are heavier than water and if the water is quiet they sink to the bottom. Stirring up the bottom increases the bacterial count; water taken from the surface decreases the count.

Good water may vary widely in the total count, as less than 25 colonies from deep source water to 100 colonies from shallow sourses. 300 or more colonies are regarded as suspicious of seepage of surface waters. The normal mean count of every season of the year is necessary. Chemical analysis showing ammonias, nitrates and chlorides indicates a possible animal contamination. Some of the organisms in animal feces are hard to differentiate from the coli group, which indicates a contamination of human excreta.

The bacterial detection of sewage is 1,000 times more delicate than chemical analysis. A form of sewage streptococcus is of diagnostic importance. This is used in England, while in the United States the detection of the coli group is sufficient to condemn the water and to say it is contaminated with sewage.

The Quantitative Analysis:

1. Shake the bottle with the sample of water 25 times, then dilute the sample with sterile distilled water, so that the dilutions are 1 in 10, 1 in 100, 1 in 500, 1 in 1,000; and if the water is heavily contaminated, higher dilutions are necessary. Immediately place the diluted water in test tubes with melted gelatin medium and others with agar medium

melted at 40 degrees C.; shake and place in petri dishes. The gelatin medium is to be placed in a dark, well ventilated incubator, with the atmosphere saturated with water at 2 degrees C., for 48 hours. The agar medium is to be incubated at 37 degrees C., for 24 hours in a dark, well ventilated incubator, the air in which is saturated with water vapor. All dishes should be inverted when placed in the incubator so that the water of condensation will not gather on the lid, or top and drop down on the medium, causing the colonies to grow together. Colonies should be counted by the aid of a small magnification glass of about 2½ diameters. If the dish is set on a ruled glass plate the counting is made easy. Counts are given in even numbers as 1,120,000 and not as 1,120,369.

If it is not desired to mix the water with the culture media, the water after dilution may be placed in the dish by lifting the lid just high enough, that the pipette can be slipped under and the contents delivered into the dish. Then the melted medium is added. The lips of all pipettes and test tubes should be flamed before pouring. Never use more than one mil of the diluted water to the dish. When using 1 mil of the sample, use 10 mils of melted medium. A porous earthen ware cover may be used instead of the glass lid, thus avoiding the turning upside down of the petri dish. The dishes with the melted media must be permitted to cool on a flat surface before incubating.

2. The Presumptive Coli Test:

Deliver into a series of Durham fermentation tubes containing lactose broth, and another set containing glucose broth, varying amounts of the water sample, as 0.1, 0.5, 1, 5, and 10 mils, incubate at 37 degrees C., for 24 hours and examine for gas. If the tube containing 1 mil of water shows gas it is reasonable to assume that the water is contaminated with coli and contained at least 10 coli to the mil. If only the tubes containing 10 mils of water show gas and the ones with lesser amounts show no gas, it is stated that the water contained coli in amounts of 10 mils only. Control tubes must always be carried, that is, tubes containing only the broth. Samples showing 10% or more gas are regarded as positive. For positive demonstration, cultures must be made from these tubes and subcultures made until the different kinds and species of bacteria are isolated and identified. Water showing coli in any dilution is suspicious and must not be used for drinking. Other tests for identification must be made, as fermentation with bile lactose broth

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ISOLATION OF SOME MICROORGANISMS FROM WATER.

1. Hoffman and Fiske medium, incubated 12 hours.
   A. Endo agar plates.
      a. Blue colonies.
      b. Dextrose, negative.
      c. Lactose, negative.
2. A. Liver broth, gas.
   B. Dextrose broth, gas.
      a. Lactose litmus agar, shake culture.
         1. Gas and acid, indicates B.coli group.
         2. No gas or acid, indicates B.cholerae suis-like organisms.
      b. Plates from B on Conradi-Drigalski's medium.
         1. Blue colonies.
            a. Dextrose, positive.
            b. Lactose, negative.
               Indicates B.paratyphosus A or B, B.cholerae suis.
               1. Dulcite, negative. Motility.
                  a. Litmus milk, alkaline. Indicates B.cholerae suis group.
               2. Dulcite, positive. Motility. Indicates B.paratyphosus A or B.
                  a. Litmus milk, acid. Indicates B.paratyphosus A.
                  b. Litmus milk, acid changing to alkaline, indicates B.paratyphosus B.
         2. Red colonies.
            A. Dulcite and lactose, positive.
               a. Adonite and gelatin both negative, indicates B.coli communis, B.coli communior, B.aerogenes group.
                  1. Saccharose, positive. Indicates B.coli communior, B.aerogenes group.
                     a. Indol, positive. Esculin bile in 6 days, positive. NaOH reaction, negative. Motility. Indicates B.aerogenes group.
                     b. Indol, variable, usually positive. Esculin bile negative. NaOH reaction, positive. No motility. Indicates B.coli communior.
            B. Dulcite, negative. Indicates B.acidi-lactici, B.lactis aerogenes group, Bact.aerogenes.
               1. Adonite, negative. Gelatin, positive. Saccharose positive. Motile. Indicates B.cloacae.
               2. Adonite, positive. Gelatin, negative. No motility. Indicates B.lactis aerogenes, B.acidi-lactici, B.aerogenes group, Bact.aerogenes.
                  a. Saccharose, negative. Indol, positive, Nitrate red, positive. Esculin bile, negative. Indicates B.acidi-lactici.
                  b. Saccharose, positive. Nitrate red, positive.
                     1. Indol, positive.
                        a. Esculin bile, in 1 day, positive. Indicates B. lactis aerogenes.
                        b. Esculin bile, in 3 days, positive. Indicates B.aerogenes.
                     2. Indol, negative. LKD
                        a. Esculin bile, negative. Indicates Bact.aerogenes.

melted at 40 degrees C.; shake and place in petri dishes. The gelatin medium is to be placed in a dark, well ventilated incubator, with the atmosphere saturated with water at 20 degrees C., for 48 hours. The agar medium is to be incubated at 37 degrees C., for 24 hours in a dark, well ventilated incubator, the air in which is saturated with water vapor. All dishes should be inverted when placed in the incubator so that the water of condensation will not gather on the lid, or top and drop down on the medium, causing the colonies to grow together. Colonies should be counted by the aid of a small magnification glass of about 2½ diameters. If the dish is set on a ruled glass plate the counting is made easy. Counts are given in even numbers as 1,120,000 and not as 1,120,369.

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which is generally more accurate than the glucose or plain lactose broth. If any coli are present, gas should form in 24 hours and not later than 48 hours. Coli and sewage streptococcus both show an acid reaction in lactose broth; hence the pink colonies on litmus agar. Waters of a fair purity rarely show pink colonies.

If no gas shows in 24 hours, the tubes are to be incubated another 24 hours, making an incubation of 48 hours. If no gas shows, the sample is negative.

Petri dishes with Endo's medium inoculated from the sample showing the smallest amount of gas, incubated 24 hours and examined. The colonies which resemble the coli are inoculated into lactose broth fermentation tubes and incubated for 24 to 48 hours. The Endo plates are replaced in the incubator for another 24 hours. Specimens are stained and examined under the microscope for the non-sporing bacillus of B. coli. Agar medium slants should also be made, incubated and examined.

If the proportion of liquefying bacteria on gelatin media is greater than 1 to 10 non-liquefying bacteria, the water is best regarded as suspicious. Intestinal bacteria as coli and typhoid do not develop rapidly at low or river temperatures and the B. coli develops less ammonia than the sewage streptococcus.

In reporting B. coli, the following must be considered:

1. Typical morphology, non-sporing, small, thick bacillus.
2. Nearly all of the young broth cultures are motile.
3. Forming 50% gas in dextrose broth, 1-3 of which is absorbed by a 2% solution of NaOH, and the remainder reported as N.
4. Non-liquefying in gelatin medium.
5. Fermentation of lactose broth with gas production.
6. Indol reaction, reducing nitrates to nitrites.
7. Reduction of neutral red with a greenish-yellow fluorescence. (Bacilli of hog cholera also have this property).

The group reactions found in water are:

1. B. coli of fecal origin, are positive in regard to methly red, indol and lactose; negative with gelatin, adonite and saccharose.

2. B. aerogenes of fecal origin, positive in regard to adonite and saccharose; negative with methyl red, gelatin and indol.

3. Aerogenes not of fecal origin, positive with indol and saccharose; negative with methyl red, gelatin and adonite.

4. B. Cloace may or may not be of fecal origin, positive with gelatin, adonite, indol and saccharose; negative with methyl red.

Contaminations found in waters as classified by Schneider are: Those which are from soils indicating surface waters and surface seepage and include the Protococcus groupe, Desmids, Diatoms, Nostoc, Oscillaria, Yeasts, molds, Spores, Pollen, and decayed vegetable tissues, these are of the Vegetable group, while those of the animal group are: Amebae, Paramecia, Spongilla, etc., Nematode ova, Animal hairs and excreta,and Insect fragments. Indications of sewage are kitchen refuse which may be divided into vegetable and animal and micro-organisms. The vegetable include starches, cereals, potato, bean, etc. Vegetable tissues from vegetables and fruits used as food, while the animal refuse includes blood corpuscles, oil and fat globules, muscle elements, fibrous elements and parasite ova. The micro-organisms are cocci forms—usually abundant, as are also a diplobacilli, streptococcus fecalis (always found in sewage, colon bacteria yeasts, mold spores and protozoa. Mineral and organic matter are results of vegetable decay, dirt and clay, sand, mineral compounds, diatomaceous earths, etc.

COMMERCIAL WATER PURIFICATION

Practically all public water supply contains bacteria, micro-organisms and suspended material, so that the water purification is for the purpose of removing these substances. It is accomplished by clarification, filtration and sterilization.

Water may be clarified by using aluminum sulphate or iron sulphate and lime.

It may be filtered by three methods: (1) Slow sand filters, which consist of beds of sand 3 to 5 feet deep, underlaid with open jointed tile or drains. The water to be treated is placed on the beds until they are well covered, and then 2 to 5 million gallons are added daily for about 3 weeks, when the sand must be cleaned by removing the top layers which contain the impurities taken from the water. These filters are usually about 1 acre in extent and are roofed. (2) The rapid gravity filter consists first of treating the water with some chemical coagulent; the water is then run into settling basins for about 8 hours. It is then flowed on sand bed filters, which are composed of strainers covered with about 12 inches

of gravel, and the gravel covered with about 30 inches of sand. These filters do not remove the bacteria. (3) Pressure filters are used in private homes, etc. They consist of a water tight steel tank, or iron case filled with the filtering material. Water is admitted under pressure. In the smaller filters a porcelain tube is placed in the iron shell. In time this tube becomes clogged with the material in the water and must be cleaned by burning, or washed by reversing the pressure.

Sterilization is accomplished by hypochlorite of lime, or chlorine gas, copper sulphate and the ultraviolet ray.

1. Calcium hypochlorite is available in two forms, compressed tablets and large containers. The tablets are made in 3 sizes containing 3, 17 and 35 mgm, of chlorine. They must be kept in a dark, dry, cool place. In using this compound, it should be rubbed with water to make a smooth paste, placed in the water to be sterilized, the container covered and permitted to stand at least 20 minutes for sterilization. The hypochlorite must be thoroughly mixed with the water to be sterilized. For treating large amounts of water, solutions of from 0.5% to 2% are used, that is about 2 to 4 pounds to 200 pounds off water. If the waters are overtreated, causing unpleasant odor and taste, the excess may be removed with sodium thiosulphate.

2. Chloramin. By adding to the hypochlorite solution just as it is taken into the water pump, a 0.3% solution of ammonia which produces chloramin, and increases the germicidal action of the hypochlorite.

3. Chlorine gas. Chlorine is fed into the water from the chlorine gas tanks, under a pressure of less than 6 pounds, for a greater pressure will cause the chlorine to freeze. The flow of chlorine is gauged by a meter, which unless kept warm will freeze. It is applied to the water by means of a water jet, which thoroughly mixes the water and gas. The flow of chlorine must be constant, using about 1 pound of gas every 24 hours to 300,000 gallons of water, or 0.000694 pounds per minute, or 0.0000115 pounds per second. The meter is constructed so that it will measure from 1-10 pound to 12 pounds in 24 hours.

Chlorine gas method of sterilization is effective for destroying bacteria, but it will not remove turbidity or color from the water. The cost of this method of sterilization is very low. For a town of 1,200 people, the cost is about $300 per year. It has been used in Lockport and Tonawanda, both towns taking water from the Niagara river, which re-

ceives the sewage from Buffalo, a few miles above. Both cities have had typhoid epidemics. In 1914 both cities installed chlorine plants and typhoid has been practically eliminated. Milwaukee used hypochlorite but on account of the bad taste and odor changed to chlorine gas with the result that in 1915 the typhoid rate was the lowest in the history of the city. Buffalo, Pittsburgh and many other cities have installed the chlorine plants with great success.

A typical filter plant may be composed of:

1. A lime saturator—6 or more tanks, each 25 feet square, in which the chemical reaction begins.

2. Two or more baffle mixing tanks, measuring 200 feet long, 25 feet wide and 20 feet deep. These figures and numbers are relative, the sizes and numbers depending upon the amount of water to be treated.

3. Settling basin, which is divided into 6 parts and having a capacity of 15 million gallons. The greater part of the turbidity and precipitated materials are removed by sedimentation.

4. Ten mechanical filter units, each with a net area of 1,000 square feet.

5. A chlorine apparatus.

6. A covered filtered water reservoir holding 10 million gallons.

In purification with alum, 2 grains to the gallon or 1 pound to 7,000 gallons of water is used. The sulphate combines with the alkalinity of the water, forming a flocculent gelatinous precipitate, consisting of aluminum hydroxide, which contains the mud, plant debris and the micro-organisms.

For softening the water, lime and soda ash are used. Lime absorbs the carbon dioxide and is converted into calcium carbonate. This happens in boiling lime-stone water or hard water, the lime forming a crust in the vessels. Typhoid and other bacteria rarely live longer than 24 hours in lime-softened water.

Insoluble coagulants. The combination of potassium permanganate 0.06 gram, manganese dioxide 0.05 gram, precipitated calcium carbonate 0.02 gram and talc 0.37 gram formed into one powder and one powder of sodium thiosulphate 0.06 gram and talc to make an equal bulk with the other powder. The two powders are added to 1 liter of water, stirred with a spoon and then filtered through cotton.

Copper sulphate 1 part to 5,000,000 parts of water is placed in a gunny sack, which is towed behind a row boat

around the edge of the reservoir. It is frequently used as an algecide, for the destruction of the blue and green algae.

Sterilization by the ultraviolet ray is efficient but too expensive for purification on a large scale. Its chief use is for the sterilization of swimming pools.

Swimming pools are also sterilized by using ½ part ozone to 1 million parts of water, and reduces the number of micro-organisms over ·99%, or calcium hypochlorite, 1 pound added at night to every 65,000 gallons of water, the pool scrubbed and flushed every day and new water added. The hypochlorite is added to the water by placing it in a perforated can which is dragged over the surface. One pool caused 236 cases of ulvovagnitis. Gonorrhoea, colds, infections from colon organisms, etc., have been traced to unclean pools.

The number of organisms are reduced in the water freezing into ice and the longer the ice is kept, the fewer the number of organisms. A count of raw water was 12,000 colonies; after making it into ice, the count was 125 colonies; another water yielded 1,400 colonies and when made into ice, 16 colonies. Although a great reduction takes place, infection may occur through handling and delivering the ice. Ice should be thoroughly washed before using in foods.

SEWAGE DISPOSAL

Sewage is a term given to the solid and liquid wastes from human habitation, manufacturing plants, houses, et al., diluted with the water used.

The composition varies during the day and night. It is decomposed by bacterial activities without odor, if there is abundant available oxygen present. This method is called "aerobic decomposition." "Anaerobic decomposition" takes place where there is a lack of oxygen and is accompanied with the production of odors.

Sewage disposal is accomplished in different ways; the easiest of which is to discharge the sewage into a body of water; this is called the "dilution method." In order that the dilution method be successful, there must be a velocity of the water current of more than three feet a second so that the solid part or sludge will not immediately sink and deprive the water of its oxygen. There must be six cubic feet per second for every 1,000 persons contributing sewage, or a dilution of at least 1 in 50. Water saturated with oxygen contains at ordinary temperatures about 10 parts in 1,000,000. The more free oxygen in the water the greater

and faster its oxidizing power. There must be sufficient diluting water in the streams to eliminate offensive odors, prevent nuisances and the spread of disease. If this is not possible, artificial purification is necessary.

To prevent discharges of floating offensive matter, screens, grit chambers or sedimentation basins are used.

Screening. Coarse screens remove floating matter objectionable to the eye and are of almost 1 by 2.5 centimeter mesh. These screens are fixed while the fine screens are mechanically operated. The fine screens have a mesh of about 0.5 by 0.05 centimeters and remove 25 parts to 116 parts per million. The coarse screens remove 5 to 10 parts per million.

Grit chambers are intended to remove heavy material as sand, gravel, cinder, etc. This must be removed on account of injury to machinery and also to prevent retarding purification. This process consists of sedimentation by reducing the flow through the basins to less than two feet per second. This process removes 10 to 40 parts per million of suspended matter.

Plain sedimentation. The sewage must stand or flow slowly through tanks where the solid matter settles to the bottom. It takes from two to eight hours for the settling to take place, and a detention of two hours removes about 60% of the suspended matter.

Chemical precipitation. One or more soluble chemicals are mixed with the sewage, forming a precipitate which drags down with it much of the suspended matter, removing 60% to 90% of it. Calcium oxide, aluminum sulphate and ferric sulphate are commonly used. With this process the settled matter or sludge must be removed from the tanks.

Septic tank. Converts the sludge in the tanks into 30% to 70% by volume of gas and reduces the solid matter weight 10% to 40% by bacterial action, and destroys in about four hours 45% to 65% of the suspended matter.

The Imhoff Tank. The sludge is digested in a separate tank from the sewage in the sedimentation tank.

Sewage may be aerated, which produces a more rapid decomposition.

Contact bed treatment.—A series of tanks filled with small pieces of broken stone, cinders or coke to a height of four feet. This material must be removed and replaced with new at least every five years. The tanks or beds are built in series, the effluent passing from one to the other, being improved in quality after each successive passage.

Or single tanks may be used; in this case the tanks are filled, allowed to stand, emptied and allowed to rest. The contact is usually one hour, the rest period is 45 minutes and six hours between fillings. Each acre of beds will accommodate 150,000 gallons for each foot of depth and removes about 60% of the bacteria.

Sprinkling filters are tanks, or beds, filled with broken stone to a depth of five to seven feet. The bottom is equipped with an underground drain system through which the effluent is drawn. The sewage is applied to the beds in a fine spray by means of a fixed sprinkler, so that the sewage is saturated with oxygen from the air. The sewage is sprayed on the beds for two to five minutes, a rest period, and then again sprayed. A sprinkler filter can treat sewage at the rate of 2,000,000 gallons a day per acre.

Intermittent sand filtration. Small volumes of sewage are applied to beds of sand four to six feet deep. 60,000 gallons per acre a day may be handled. It removes about 99% of the bacteria, and the effluent is free from odor and turbidity. Intermittent application of the sewage is essential so that there is sufficient oxygen in the pores of the material to carry on the oxidation process.

Broad irrigation disposal consists of intermittently flowing 3,000 to 12,000 gallons of raw sewage a day per acre on cultivated land. This process causes bad odors, and it is objectionable to use untreated sewage on growing vegetables; also there is a liability of the communication of disease by flies, insects and by the vegetables.

Activated sludge is produced by the aeration of sewage in tanks with sludge from previously aerated sewage and requires two cubic feet of air per gallon and four to five hours aeration. There must be sufficient oxygen and sufficient agitation in the tanks so that the acivated sludge comes in contact with the sewage. This process has a possible commercial value, for the sludge is used in Milwaukee and other cities as a fertilizer.

Sterilization of sewage is accomplished by hypochlorite of lime or liquid chlorine, requiring for crude sewage four to 12 parts of chlorine per million parts of sewage. Septic effluent, 10 to 15 parts to one million parts of sewage, and by the sprinkler filter method three to four parts per million of sewage. The contact time should be at least 15 minutes and it should remove 95% of the bacteria.

Sludge is disposed of by: Digestion and then drying in the air; drying by pressure the chemically precipitated

sludge; applying it to land; lagooning—applying the wet sludge to land by dykes; dispersion into harbors, lakes or rivers; filling low lands; incineration; mechanical drying; and as filters for fertilizers.

A typical sewage disposal plant consists of: Grit chambers for the removal of sand, cinders, etc.; Imhoff tank to remove suspended matter and permit sludge digestion so that it may be dried on sludge drying beds without causing nuisance; sprinkle filters for oxidizing organic matter in solution; and basins for sedimenting the filtered effluent. Or removal of the suspended matter with fine screens and intermittent sand filtration.

The souring of milk is caused by lactic acid which is produced by the breaking down of milk sugar (4% to 5% in normal milk) by the action of various micro-organisms. The milk is at first sour but when the lactic acid reaches 0.7% to 0.9% a curdling results, causing the casein to be precipitated and the milk changing from a slight alkaline to acid reaction. The sugar destruction, bacterial growths and acid production are so closely allied that explanation is difficult. The old theory of milk souring caused by thunder storms has proven a fallacy. Unless there are micro-organisms present souring will not take place. A great many varieties of organisms enter into the souring of milk, among which are normally found:

The lactic acid bacteria, which are divided into two classes; the one is capsulated and gas-forming as the B. lactis aerogenes. These organisms are closely related to the B. coli but do not have the motility or the power of producing gas from potato starch. To the other class belongs the Bacterium acidi lactici or Streptococcus lacticus. This organism is on the skin of cows and in cow dung. It resembles in appearance the Streptococcus pyogenes.

Isolation.—A sample of milk (not curdled) is diluted 1 in 1,000 with sterile water and plated on litmus gelatin and incubated at 20 degrees C., for two days. The colonies appear as minute red spots, gradually enlarging and generally growing just under the surface of the medium.

Streptococci and other cocci usually come from sore or irritated udders. The lactic acid group comes from the air and dust and hair of the cow.—Bacterium aerogenes and other gas producers from the external parts of the cow; the coli group from the fecal contaminations of the cow, or from the hands of an uncleanly milker. Diphtheria and typhoid from the uncleanly milker. Yeasts and molds come from the air, the milker, milk pails, wash water, et al.

Bacterium aerogenes is not so common but is widely distributed, and when plated on litmus gelatin medium they appear as round raised, distinct red colonies. The bacterium is motile, large and much longer than broad. It is the chief gas producer found in normal milk and is often the cause of the spoiling of cheese.

Peptonizing and rennet forming bacteria. Milk may curdle and yet not sour. This is called "sweet curdling," occurring generally in the morning milk in the fall of the year. Sweet curdling is due to a certain class of bacteria,

which have the power of secreting certain chemical ferments, very similar to those produced by the digestive glands of our stomachs and intestines. When these bacteria are plated they appear as pits in the gelatin medium and grow larger as they liquefy the medium. They liquefy gelatin but not agar medium. When milk is inoculated with these bacteria the milk is curdled within 24 hours and usually the curdling is without acid production. The curdling is caused by bacterial secretions, similar to rennet. These ferments are called enzymes. The converting of casein and other proteids into a soluble form is caused by "peptonizing bacterial secretions."

The process of digestion occurs when the bacteria first curdle the milk, then the curd or casein is softened and converted into rennet-like bodies. Some species of bacteria produce both acid and ferments. These are the cause of butter and cheese becoming bitter. Butyric acid production and other fermentations also take place in milk.

Milk may contain only two colonies of bacterial growth to the mil, and these may be B. typhosus, and are dangerous; or milk may contain 100,000 colonies to the mil, and they may not be dangerous; so it is necessary to determine not alone the number but the kind of bacteria in the milk. The number varies according to the season, the method of milking, handling, etc. One specimen of milk contained in the summer an average of 50,000 colonies, and in the winter 750 colonies. Milk should have very few bacteria and show no sediment on standing. Full milk should have 3½% butter fat, skim milk contains no cream, and half milk contains 1.75% butter fat, and should contain not over 500 leukocytes to the field of centrifuged milk; although in one case in the summer, milk from an apparently healthy cow showed 10,000 leukocytes; such milk is not fit for human use. If milk is produced in a sanitary manner and in the proper way, no preservatives are needed. Formalin, salicylic, benzoic and boracic acids are the most common preservatives used. In England 0.4% benzoic acid is permitted. In the United States no preservatives of any kind are permitted.

Many cases of epidemics have been traced to milk as the source of infection; as diphtheria in Massachusetts in 1916; scarlet fever, sore throat in Chicago; and typhoid in New York city. About 5% of the epidemics of scarlet fever and diphtheria are due to infected milk.

Some of the organisms in milk are called "neutral bacteria," that is those which have no part in the dairy prob-

lems. Any colonies on litmus gelatin which are not acid producers, and appear as whitish or gray spots on or under the surface have little or no action on the milk. Butyric acid fermentation, rancid odors in butter and cheese, are produced by some of the organisms which do not have time to develop in the milk. For the isolation of the butyric acid bacteria, milk is boiled for two minutes and plated under anaerobic conditions. The boiling kills nearly all of the bacteria, except the spores of the butyric acid bacteria.

Abnormal bacteria in milk produces many changes as:

Slimy milk. This is very common and may be of two kinds; slimy at the time of drawing or milking, caused by diseased udders and attributed to Garget, and usually accompanied with blood from the diseased parts. Such milk is unfit for use. The second kind is where in 12 to 24 hours after drawing, the milk becomes viscous and then extremely slimy, so much so that it can be drawn out in threads. This is commonly caused by Bacterium lactis viscocus. In many cases it is prevented to some extent by other organisms of the lactic acid group. It may also be caused by bacillus aerogenes, under certain conditions. One diary has infected whole communities through the creamery. The organism causing slimy milk is carried by dust, milk cans, milkers, etc. The milk is unfit for use, although it is considered a great delicacy in Norway. The remedy is cleanliness and disinfection, washing the cow and especially the udders, whitewashing and cleaning the barns. Slimy milk is used in Holland for the production of Edam cheese.

Ropy milk is caused by Streptococcus hollandicus. The same precautions should be observed as given under slimy milk.

Bitter milk may be caused by the cow eating certain plants as rag weed, or it may be caused by Bacillus liquefaciens amari, torula amara and other bacteria.

Bacteria from the udders.—Externally almost any kind, internally, if the cow is not diseased and after the first milk or fore-milk has been drawn, there should be a few or no bacteria in the milk. Milk taken under aseptic precautions and without any preservatives and kept cool has been transported from Chicago to Europe without becoming sour or losing its wholesomeness.

Alcoholic fermentation in milk is caused by yeasts. Cane sugar is added to condensed milk as a preventative.

Koumiss is made from mares' milk by the Tartars, by using a small quantity of old koumiss as a starter, added to fresh milk. This is an alcoholic fermentation.

Kefir is an effervescing sour milk made by the Caucasus inhabitants from cow, goat or sheep milk. A starter is used consisting of the kefir grains which are granular, irregular and gelatinous. These fermented milks are produced by a number of different kinds of micro-organisms which are contained in the starters, which have been handed down from time immemorial, from generation to generation.

Blue milk (not watered milk) has been studied 70 years. The milk after standing for several hours changes to a bluish tint, caused by Bacillus cyanogenes (and sometimes Bacillus pyogenes); this is not common to the United States. Certain fermentations may also change the color.

Red milk may be caused by blood, or by Bacillus prodigiosus or Bacterium erythrogenes, or Bacterium lactis rubefaciens and others. Seldom seen in the United States.

Yellow milk is caused by Bacillus synxanthus.

Black, brown, green and various colored milks may be produced by the various chromogenic bacteria.

Tainted milks may be caused by Bacterium lactis foetidus, or by vegetables as turnip-tasting milk caused by the cow eating turnips, etc.

Soapy milk may be caused by Bacterium sapolactilum, or Bacterium lactis saponacei.

Odium lactis is widely distributed, and is half way between bacteria and molds. The growth at length produces chemical decomposition and strong odors and flavors, as rancid butter and cheese.

Micro-organisms commonly found in milk are the various lactic acid bacteria such as the Bacterium acidi lactici, or the Streptococcus lacticus, Streptococcus progenes, Streptococcus aureus and albus, bacteria of the colon group, molds, yeasts, Bacillus pyogenes, Bacillus subtilis, Bacillus prodigiosus, Bacillus aerogenes capsulatus, etc.

If a cow is absolutely healthy, few or no bacteria are found in the milk, but as the cow is prone to disease, in nearly all cases bacteria are found in the milk ducts of the cow. The organisms causing consumption, foot and mouth disease, mastitis, garget, diarrhoea, streptococci sore throat and many other diseases communicable to man are found in the cow and milk. In many cases most of the bacteria come from the milking, or subsequent handling of the milk.

192 samples of milk obtained from five dairies, gave counts as high as 265,000 colonies to the mil, and on isolation showed bacilli, micrococci, streptococci and staphylococci; and in 113 samples pyogenic staphylococci (Some of

those which produce boils and carbuncles) were found. Bacillus abortus was found in 45 samples; Bacillus abortus, variety lipolyticus, was present in 45 samples.

Aseptic milk.—Milk drawn with exceptional precautions to remove as far as possible all bacterial contamination. The flanks and udder of the cow are washed and dried; the barns must be clean; a special place provided for milking; all milk vessels must be sterile; and last but not least a clean milker. External conditions as the air, vessels, dirty hands of the milker, milking the cow while standing on manure, and unsanitary vessels and subsequent handling are some of the causes of bacterially dirty milk.

Several tests showed that the number of colonies of growth from the milk at the udder ranged, in 20 cows, from none to 400 per mil, after straining from 20 to 60,000 and after cooling averaged 54,000, in the tank below the cooler averaging 175,000, and after bottling ranged from 80 to 800,000 colonies to the mil: this shows the external contamination.

Ordinary temperatures tend to aid in the multiplication of the bacteria. Fresh milk should not show over 100 colonies to the mil and no pathogenic bacteria. If the milk is placed on ice immediately after milking, for 24 hours, it will show fewer colonies than at the time of milking. A sample of milk was counted and showed 9,765 colonies; it was divided and one half placed at 20 degrees C., for two hours and plated and the count showed 8,645 colonies; the other half was plated at 37 degrees C., for two hours and the count showed 8,486,000 colonies per mil.

Bacteria grow slowly if at all in frozen milk; a slow growth is noticed several degrees above freezing; but for three or four days no appreciable increase in growth is noticeable. An abundance of growth was obtained at 20 degrees C., and at 37 degrees C., rapid growth, but at 50 degrees C., very little lactic acid bacterial growth; and at 60 degrees C., lactic acid growth practically ceased, but acid producers, typhoid, diphtheria and organisms of this nature grew rapidly at 37 degrees C., while there was a retardation of lactic acid organism growth. In milk at the proper temperatures, the lactic acid organisms grow so rapidly that an almost pure culture may be obtained, to the exclusion of almost all other bacteria.

Toxin poisoning from milk products. Milk, cream, cheese and ice cream may produce a toxin from standing too

long in cans, especially dirty cans. Cheese may produce a toxin called Tyrotoxicon.

Clean milk production depends upon the health of the cow, the condition of the stables, milking rooms, milk vessels, the food of the cow, and the condition of the milker; moistening the flanks and udder of the cow, using milking machines, rejecting the fore-milk and strippings, proper cooling, aeration, filtering or centrifugal methods of cleaning the milk; milking directly into covered strainer buckets, preventing infections from the creamery, barn and dairy by cleanliness and proper disinfection, proper treatment of the milk for market, transportation and cooling and a bacterially clean milker.

A bacterial count of the country air showed an average of 48 colonies to the cubic foot (about 28 liters) of air. The city showed: Street air, on a quiet street, 92; on a dusty and windy street, 600; in a room with closed windows, 118; in a laboratory, 92; and in a factory, 214. In the air of a milk room there were 840 colonies to the liter of air; in the milk, milked into a covered pail, 40 colonies; in milk milked in an open pail, 322; and sterile water poured from a bottle into a covered pail in the milk room, 21 colonies. The air in the milk room just after the cows had been taken out showed 2,200 colonies, the increase being due to the air set in motion by the movement of the cows. In one test of the number of colonies from a milker's hands, there were over 4,000 colonies.

Sterilization of milk. The milk is heated to a temperature to destroy all bacteria. This is accomplished under steam pressure. Boiling milk in the air causes certain chemical changes which interfere with digestion, and when taken for some time may cause scurvy and constipation in infants.

Pasteurization is accomplished in two ways. The flash pasteurization in Pennsylvania consists of heating the milk to 81.1 degrees C., and in Michigan to 85 degrees, and chilling the milk at once. This method is little used. The "holding pasteurization" method is heating the milk to 62.7 degrees C., for 30 minutes (Michigan law). This is accomplished in several ways: By passing the milk through pipes in a steam boiler; or placing the milk in a tank through which steam pipes are coiled and keeping the milk in motion by mechanical agitators. This process is considered safe and desirable. It kills about 99% of the bacteria, including Bacillus tuberculosis, Bacillus typhosus, the organisms causing

scarlet fever, those of malta fever and the foot and mouth disease and various streptococci and staphylococci and practically all the non-sporing organisms pathogenic to man.

Milk is also pasteurized by the holding process after it has been placed in the bottles and then gradually cooled.

Certified milk is milk which is certified as to the production under the following heads: 1. Veterinarian inspection; 2. Washing and sterilizing the vessels; 3. Cleansing of the cow, hair kept clipped short on flanks and udder; 4. Covered milk pails; 5. Covering cans tightly when filled—Under our laws cans must have no seams or dents; 6. Clean milking rooms; 7. Immediate cooling, after milking, as low as possible without freezing; 8. Clean milkers; and 9. Proper subsequent handling.

Avoid danger. Four rules for all to follow: 1. Never take the original milk bottle into the sick room. If the house is under quarantine, have the milk man pour the milk into your container. 2. Never let the milk stand uncovered. 3. Never permit anyone, well or sick, to drink from the bottle. 4. Never return the bottle unless it has been washed with soap and water and scalded—There is a State law compelling you to do this, under a heavy penalty.

Condensed milk was first made by Borden at White Plains, N. Y., in 1856. The Ekenburg process is now used. It consists of spraying milk under pressure on the inner side of a heated drum, heated to about 166 degrees C., in a partial vacuum. The common method is to spray the milk against a highly heated revolving cylinder, the milk being instantly dried and scraped off and forms the "milk powder" or "dried milk." Milk which has had only part of its water removed forms the ordinary condensed milk. This may be unsweetened or sweetened by adding sugar. These are also called concentrated milks. There is an excellent milk powder on the market which is made by forcing the milk under great pressure into a chamber of moving hot dry air, the dry air absorbing the water and the dried milk falling to the bottom. This preparation is put on the market in various size packages; the one-pound package of full milk (with the cream) will when mixed with water make four quarts of milk containing over the required amount of butter fat, and the one-pound package of skim milk will make five quarts of milk with 3.5% butter fat. This is a convenient way to buy milk. It avoids loss by souring and is practically bacteria-free.

52 samples of commercial condensed milk were examin-

ed and all found to contain bacteria; 80% contained the various staphylococci as aureus albus and citrus; 70% contained various streptococci as the pyogenes; and the average count of all bacteria was 1,000,000 colonies per mil. Lactic acid organisms, those of the coli group, yeast and molds, were also found and isolated.

In 1917 there were over 300 establishments in the United States devoted to condensing milk. They used daily 15,000,000 pounds of milk, with a value of $33,000,000. In 1880 the average price paid per pound for the milk was 12 cents; in 1890, 9 cents; and in 1916, 10 to 12 cents.

The requirements of the American Milk Association are: The milk must contain 3.5% to 4.5% butter fat and proteins 3% to 5%; certified cream must have 16% to 20% cream. No preservatives or coloring may be added. The gravity must be between 1.029 and 1.034 and contain less than 10,000 colonies of bacterial growth in the summer (too high—should be 100) and no pathogenic bacteria present. Certiled milk not over 3,000 colonies. The standards of some cities are not over the following number of colonies to the mil: Chicago, 500,000; New York City, 350,000, and certified milk, 10,000; Baltimore, 500,000, Boston, 500,000; Philadelphia, 10,000; Milwaukee, 250,000; Montclair, N. J., and Rochester, N. Y., 100,000. In 1913 in Springfield, the milk averaged 577,000 colonies; but by observing sanitation the count has been lowered to less than 46,000 colonies. These requirements are all too low; milk can be produced with not over 100 colonies.

Philadelphia requirements are:

Class A milk must have a bacterial colony count of not over 10,000 per cubic centimeter, with no disease-producing organisms or peptonizers, and must come from tuberculin tested cattle with a dairy scoring of not less than 90 out of a possible 100 points on the score card, which entitles to the rating of an "excellent dairy."

Class B milk must have a bacterial colony count of not over 100,000 per cubic centimeter with no disease-producing organisms or peptonizers, and must come from healthy cattle with a dairy scoring of not less than 80, which is required for the rating of a "good dairy."

Class C milk must have a bacterial colony count of not over 500,000 per cubic centimeter with no disease-producing organisms, and must come from healthy cattle with a dairy scoring of not less than 70, which is rated as a "fair dairy."

Class D is pasteurized milk having a bacterial colony

count of not over 1,000,000 per cubic centimeter before pasteurization and not over 50,000 per cubic centimeter after pasteurization with no disease-producing organisms.

Class E includes all raw milk with a bacterial colony count of over 500,000 from dairies with a score card under 70.

Certified milk from farms having the certificate of the Philadelphia Pediatric Society forms the sixth class, and is practically of the same grade as Class A milk.

The grading of dairies is done by the milk hygiene agent of the board, a trained veterinarian, and the bacteriologic and chemical examinations are made by the state livestock sanitary board laboratory and a commercial laboratory in Philadelphia, samples being collected and shipped by the milk hygiene agent or the health officer. Reports are made to the board quarterly, the classification in such reports being based on three or more surveys and milk examinations during the quarter. In case less than three surveys have been made, and this happens only with the new producers, the dairy is reported as unclassified. These quarterly reports are published as news by the local weekly paper, and many consumers are influenced in the choice of a milk dealer.

STANDARDS FOR MILK AND CREAM

Food inspection decision 178, of the United States Department of Agriculture, issued April 17, 1919, promulgates the following definitions and standards for milk and cream, as adopted by the joint committee on definitions and standards, July 30, 1817:

1. Milk is the whole, fresh, clean, lacteal secretion obtained by the complete milking of one or more healthy cows, properly fed and kept, excluding that obtained within fifteen days before and five days after calving, or such longer period as may be necessary to render the milk practically colostrum free.

2. Skimmed milk is milk from which substantially all of the milk fat has been removed.

3. Cream, sweet cream, is that portion of milk, rich in milk fat, which rises to the surface of milk on standing, or is separated from it by centrifugal force. It is fresh and clean. It contains not less than 18 per cent. of milk fat and not more than 0.2 per cent. of acid-resisting substances calculated in terms of lactic acid.

4. Whipping cream is cream which contains not less than 30 per cent. of milk fat.

5. Pasteurized milk is milk that has been subjected to a temperature not lower than 145 degrees F., for not less

than 30 minutes. Unless it is bottled hot, it is promptly cooled to 50 degrees F., or lower.

6. Buttermilk is the product that remains when fat is removed from milk or cream, sweet or sour, in the process of churning. It contains not less than 8.5 per cent. of milk solids, not fat.

7. Homogenized milk or homogenized cream is milk or cream that has been mechanically treated in such a manner as to alter its physical properties, with particular reference to the condition and appearance of the fat globules.

For the laws of this state, see the "Model Milk Ordinance," copies of which may be obtained from the Department of Health, Harrisburg, Pa. This ordinance divides milk into 5 classes as follows: 1. Certified milk. 2. Grade A milk (raw) is practically the same as certified milk and shall develop not more than 100,000 colonies of bacterial growth to the mil and cream not over 500,000 colonies. This grade of milk should not show over 500,000 individual bacteria per mil when counted by direct observation under the microscope. 3. Grade A milk (pasteurized) shall develop not more than 50,000 colonies and cream not over 250,000 colonies per mil, this milk before pasteurizing must not contain over 500,000 colonies and not over 2,500,000 bacteria when counted by direct microscopical examination. It must be delivered within 24 hours after pasteurization. 4. Grade B milk (pasteurized) must contain less than 1,500,000 colonies and less than 7,500,000 bacteria when counted direct per mil, before pasteurization. After pasteurization, it should contain not over 100,000 colonies and not over 500,000 bacteria by direct count, per mil. This milk must be delivered within 36 hours after pasteurization.

5. Grade C milk (pasteurized) shall not contain over 300,000 colonies and cream not over 1,500,000 colonies per mil. This milk must be delivered within 36 hours after pasteurization and stored and handled in containers of not less than 1 gallon capacity.

All the milk containers except Grade C must contain specified lettered caps, and Grade C must have "Grade C" painted on the containers.

Pasteurized milk is milk held for 30 minutes at a temperature of 145 degrees Fahrenheit.

Ice cream. Boston has a standard of not over 500,000 colonies to the mil. Montclair, N. J., reduced the number from 35,000,000 to 1,000. Ice cream made in a sanitary way from clean milk should contain few or no colonies.

Bacterial count in Ice Cream. Samples are collected in sterile 4 oz. wide mouth glass stoppered bottles. After the material has liquefied in the bottles, 1 mil is diluted with 99 mils sterile water, thoroughly mixed by shaking and a second dilution is made, thus making a dilution of 1 to 10,000. Then 0.1 mil is inoculated into a Petri dish containing 10 mils liquefied agar at a temperature of 45° C. mixed with a rotary motion, permitted to harden and incubated. Another Petri dish is inoculated with 1 mil of the 1 to 10,000 dilution, incubated at 37° C. for 48 hours. Then the colonies are counted with a 2½ diameter (3½x) lens, multiply the count by the dilution will give the number of colonies to the mil.

Cream ripening is caused by certain micro-organisms chiefly of the lactic acid group, in 12 hours or less. A standard has been set which is: No B. coli in less than 19 mil quantity in unripened cream, and not more than 5,000,000 colonies, or in ripened cream not over 150,000,000 colonies to the mil and no pathogenic bacteria.

Buttermilk contains about 250,000,000 colonies per mil.

A sample of butter when two hours old contained 50,000,000 colonies; 24 hours old, 25,000,000; 48 hours old, 2,500,000; and 50 days old, 300,000 colonies to the gram.

Butter made from "starters," which are cultures of bacteria, is always uniform in odor and taste. The starters are composed of about 95% lactic acid organisms and 5% other bacteria. The "off flavors" are caused by slow oxidation of the non-fatty constituents. The amount of "off flavor" is in direct proportion to the amount of acid present in the cream.

In a sample of old butter there were 1,400 colonies per mgm. of butter, and in a sample of fresh creamery butter there were 635,000,000 colonies per gram.

In one sample of milk, after cooling, cultures showed 16,000 colonies; after standing at room temperatures for 24 hours there were 280,000 colonies. A sample of milk milked under aseptic conditions and incubated at 37 degrees C., showed 12 colonies, and counts made every 12 hours for four days showed an increase for every 12 hours of about four times the number of colonies for the previous 12 hours, until the third day, when there was a decrease in the number of colonies. Another sample of milk showed at the farm, 20,000 colonies; at the dealers, 53,000 colonies; and when delivered to the consumer, 360,000 colonies.

Cheese is divided into two classes, hard and soft. The hard cheese is made by the rapid curdling of milk, and the

whey is separated from the curd. The soft cheese is curdled slowly and much whey remains in the curd. Cottage cheese is unripened curd. The soft cheeses are Brie, Camembert, Gorgonzola, et al.

Brie and Camembert cheese ripening is caused by molds growing on the surface; Roquefort, Gorgonzola and Stilton are ripened by the molds penetrating the whole curd. Penicullium glaucum (blue-green) and candidum (white) mold are concerned in the ripening of Camembert cheese. Swiss and Belgian cheese ripening is due to Oidium lactis; Roquefort cheese, some of the Penicilliums.

The hard cheeses as Cheddar, Swiss, American, Edam, et al., are ripened by bacteria.

The peptonizing of the curd is due to one of the aerobic casein dissolving bacilli of the Bacillus subtilis group, probably the Tyrothrix, and also some casease-producing cocci; these two are concerned in the cheese ripening.

The rusty spots on cheese are due to Bacillus rudensis.

Microscopic Test of Pasteurized Milk. Add slowly, to prevent coagulation, 1 part of a saturated aqueous solution of methylene blue to 5 parts of milk; stand for 15 to 30 minutes; then centrifuge. Place the sediment on a slide, air dry and examine. Raw milk shows the whole field stained light blue; the depth of the stain depends on the thickness of the sediment on the slide. Clear areas are seen, the smaller are fat; the larger, fat clusters and if nuclated, are leukocytes, which under the lower power are practically colorless except the mononuclears which are stained. Heated milk appears as little, if any, stained background except very thick parts, which may be blue. Leukocytes are stained and irregular in outline, appearing shrunken—this depends upon the amount of heat used and also the depth of the stain. Nuclei are stained. Pasteurized milk shows stained leukocytes with altered shapes and shrunken less than one-half normal size. Controls should be carried along with the test.

Raw or Boiled Milk Test. The reagent is an alcoholic solution of methylene blue 5 mils, 40% solution of formaldehyde 5 mils, distilled water 190 mils. 1 mil of the reagent is added to 20 mils of milk, then warmed on a water bath for 10 minutes at 45 degrees C., and examined for color. Raw milk is decolorized; boiled milk is blue.

Oleomargarine and Renovated Butter Test. Melt a small piece in a spoon, stir with toothpick. At a brisk boil there is a noisy spluttering, and no foam; genuine butter is just the reverse.

Fat Cohesion Test. Fill a small beaker half full of skimmed milk, heat to near boiling, then add 5 grams of the sample, stir over the flame until melted; remove and place beaker in ice water; when it begins to congeal, stir with a toothpick. Fats or oils collect in one mass or lump at the end of toothpick; the fine butter granules do not adhere.

Catalase is measured by collecting over water the oxygen given off in 2 hours, when 15 mils of milk is mixed with 5 mils of ½% hydrogen dioxide at 20 degrees C. The amount should not exceed 3.5 mils for potable milks. Colostral milk and milk from infected glands run much higher.

The reducing power of milk is measured by adding 20 mils of milk to 5 drops of a solution containing 1 gram methylene blue in 40 mils of 50% alcohol. Milk of a very poor quality loses the color in 15 minutes but milks containing a very few bacteria remain colored 5 or more days.

CULTURING THE BACILLUS BULGARICUS

From an examination of a number of commercial products, it was found that few on the market were viable. The bacillus will ferment glucose and lactose, producing much acid; this acid kills the organism and thus many cultures are killed, so that most of the preparations contain few viable bacillus unless they are fresh and preserved on ice. Broth cultures contain more viable bacilli than the other forms—many preparations were heavily contaminated, some contained B. coli. The preparations should be numbered as to the number of bacilli they contain and should be given a safe date of expiration. The bacillus to be of value to man must be taken in enormous quantities. The bacillus occurs normally in feces of man, cows and horses.

The uncertain results obtained in Metchnikoff's lactic ferment treatment are due to the insufficiency of food for the organisms, especially when abnormal fermentation takes place in the large intestine. Starchy food must be administered along with the large amounts of lactic enzymes. To enable the starch to reach the large intestine, unaffected by the digestive juices, the starch may be coated with paraffin with a melting point of 54 degrees, in the proportion of 1 part paraffin to 5 parts of starch. The dose of this prepared starch is 50 gms. taken in two doses together with the lactic culture.

Make your own cultures and give your customers a pure fresh culture. The bacillus is easily grown on milk whey—Counted as in the making of bacterial vaccines and prepared every day.

TESTING COMMERCIAL CULTURES FOR VIABILITY

¼ L. milk is pasteurized, cooled and 2 to 3 tablets of the contents of the tube of culture is added, incubated or kept warm at about 37 degrees C., for 12 hours; if the cultures are viable the milk will be curdled with a sharp acid taste and with no gas bubbles or separated whey. The manufacturers place the time within which the preparation should be used on the package and the package should be exchanged for fresh ones on or before this expiration date.

MAKING THE PREPARATIONS

The Bacillus Bulgaricus is a non-pathogenic, non-motile, non-sporulating, non-liquefying, Gram positive bacillus (involution forms may be Gram negative), varies in length from 2 to 50 microns and occurs singly or in short or long chains —strepto bacillus.

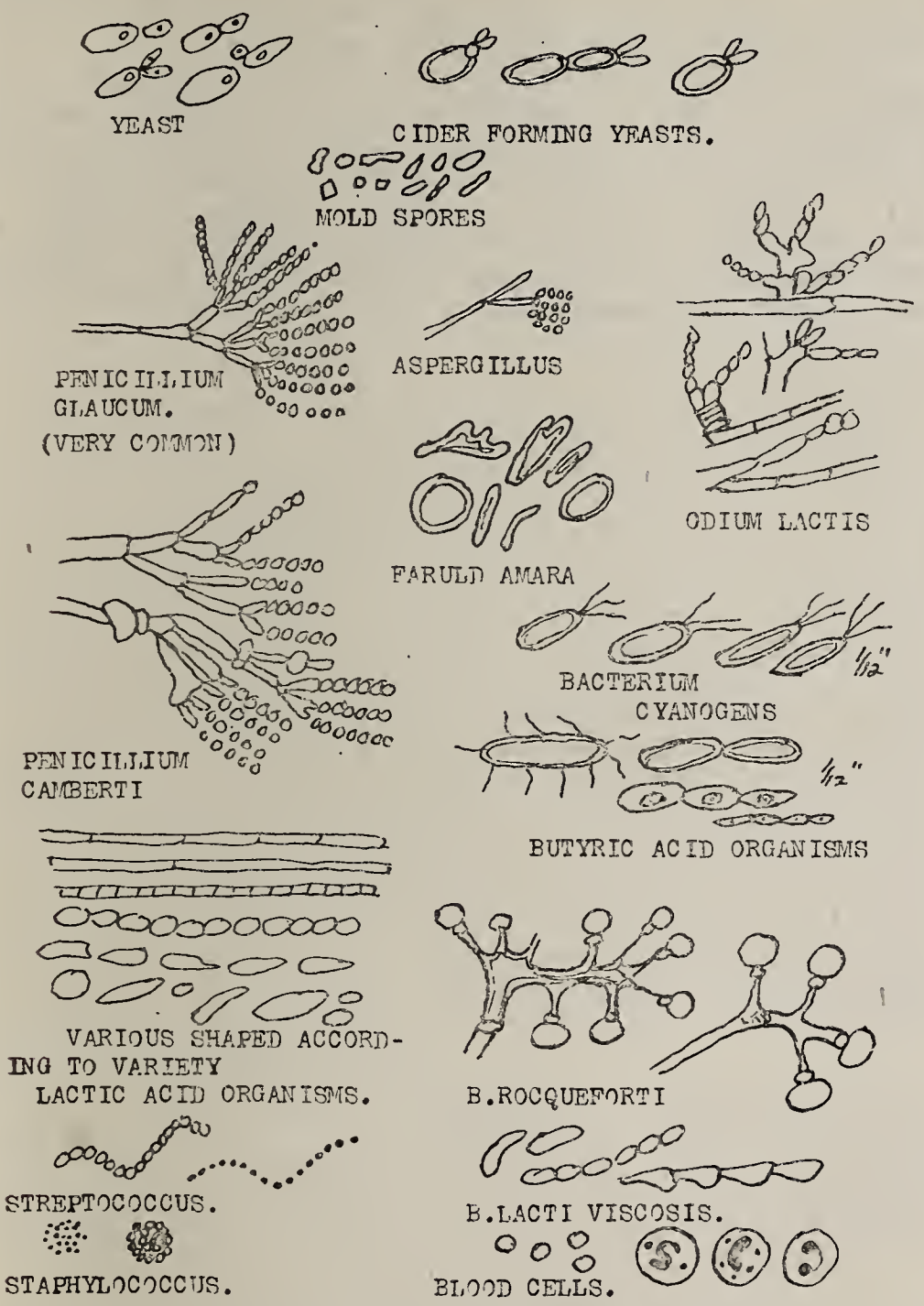
It is both aerobic and anaerobic, growing with difficulty on ordinary media, cultivated easily in milk or in mediums containing milk, milk whey or malt. Growing best at 44 degrees C.; fair at 30 degrees C.; slight growth at 25 degrees C.; growth ceases at 20 degrees C.

½ L. milk from several sources is mixed and allowed to stand in a warm place until curdling takes place. Place from this starter, about 2 mils into milk which has been pasteurized by being in live steam or water for ½ hour, and cooled. Permit to stand in a warm place for 8 to 10 hours; a smooth curd should form, without whey or gas bubbles. If gas or whey and a straw colored liquid are formed, it indicates a mixture with other organisms. When a pure culture is obtained it must be propogated from day to day; one L of starter is sufficient to inoculate 60 to 120 liters of milk.

The curd should be churned or stirred so that a smooth preparation is obtained, and afterward placed on ice and not kept longer than 24 hours. Cultures may be purchased on the market and added to pasteurized milk. Some manufacturers add to the finished preparation lemon juice, sugar, chocolate, etc., to make a more pleasant drink. Unless the preparation is kept cold, the curd contracts and becomes tough.

The commercial product appears in tablets, which are made from the milk or liquid cultures mixed with milk,

sugar, starch or other, dried and pressed into tablets. Tested by dropping one tablet in 1 liter sterile milk and incubated, it should cause souring in 24 hours.



NEARLY ANY KNOWN MICRO-ORGANISM MAY BE FOUND.

MICRO-ORGANISMS FOUND IN MILK.

Preserved by drying at a low temperature and mixing with inert substances as petrolatum; this process is patented; and a red color is added to minimize the effect of light on the culture. It is a question if the coating will not prevent proper multiplication in the intestines, although this method will keep the culture viable for a long time.

Probably the best method is the broth culture or liquid culture. To obtain a stained slide of the culture the culture must be mixed on the slide with a drop of the white of egg; on account of the lack of albumen in the bacillus walls they will not adhere to the slide unless albumen is added. The viable organisms fixed on the slide by this method and stained by Gram stain are all Gram positive, while the dead organisms are Gram negative.

EXERCISE

Examine a centrifuged specimen from 10 mils of milk. Then stain by the Gram stain and note the number of leukocytes to the microscopic field. There should not be over 12 or a total of 200,000 to the mil. Streptococcus should not be present, if over a total of 4 bacteria to the field, the milk should be rejected. 20% to 50% lactic acid organisms and large number of gelatine-liquefying bacteria are also a cause for rejection. Make a differential count of at least 200 of the cellular elements.

DIRECT MICROSCOPICAL EXAMINATION

Measure accurately one-hundredth of a cubic centimeter of milk or cream by means of a sterile capillary pipette and spread evenly on a slide with a ruled area of 1 square cm. The slide is quickly dried in a warm oven protected from dust; it is then immersed in xylol or other fat solvents for one minute or longer; drained and dried, and immersed in 80% alcohol for one minute or more. Then drained and immersed in a fresh saturated aqueous solution of methylene blue for one minute; washed with water and decolorized in alcohol until the background is a pale blue. The slide dried and examined under the microscope, the number and shapes of organisms present are counted. A special eye piece is used, which is made by inserting a diaphragm with an opening of 8 mm, in diameter and cementing cross hairs over the opening. The draw tube is adjusted so that the diameter of the opening in the eyepiece measures 0.146 mm. on the stage. Then the number of bacteria seen in one field multiplied by 600,000 will equal the number in one mil of milk, or the total seen and counted in 30 fields multiplied by 20,000 will equal the total number in one mil. All bacteria are to be counted including those which are in the leukocytes and those which appear as dividing are counted as two. Several slides should be made and 30 fields on each slide counted—the oil immersion lens should be used.

DETERMINING THE NUMBER OF STREPTOCOCCI

Dilutions of 1, 0.1, 0.01, 0.001, et al, are made of the milk with sterile water and tubes of glucose neutral red broth are inoculated and incubated at 37 degrees C. for 48 hours, and then examined in the hanging drop for chain formation.

ISOLATION OF THE STREPTOCOCCI

Diluted loop-fuls of the neutral red glucose broth are streaked across agar medium plates and incubated for 24

hours at 37 degrees C. The streptococcus are then subcultured in broth and streak cultures are made in agar medium tubes, and the following points noted: Morphology, growth on streaked agar medium and in broth, growth on gelatin slopes, action upon litmus milk, the production of acid in lactose, saccharose, salicin, mannite, racinose and inulin.

LEUKOCYTE DETERMINATION

One mil of milk is centrifuged with 10 mils of Toissen's solution for 10 minutes; the cream is broken up with a glass rod and again centrifuged for 5 minutes; 10 mils of the fluid are carefully removed, the remaining 1 mil containing the deposits is well mixed with the platinum needle and a count is made with the hemocytometer as directed under a differential blood count.

A total bacterial count is made in the same way as a total count is made as directed under water analysis.

ARTIFICIAL MILK

To 100 liters of water at 80 degrees C. add 25 grams potassium phosphate or sodium phosphate, and sugar sufficient to make 4.5% and 45 kgs, or peanut or soya bean meal. Boil in a steam jacketed pan, cool and inoculate with a mixed lactic acid bacteria culture until the required acidity is acquired, pasteurize, cool and add 0.08% citric acid. The cream is added in the form of cocoanut oil or one of the other bland tasteless nut oils. It is then evaporated in vacuum pans to milk consistency. This is said to produce a wholesome milk which answers all milk requirements for proper nourishment and also may be used in cheese making.

CONSTITUENTS OF MILK

	Woman	Cow	Ass	Mare
Water	89.08	87.02	91.65	90.71
Casein	1.52	4.43	1.32	1.20
Butter Fat	3.55	3.15	0.11	1.71
Milk Sugar	6.50	4.77	6.03	5.70
Salts	0.45	0.65	0.34	0.73

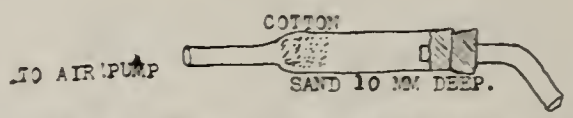
BACTERIAL ANALYSIS OF AIR AND SOIL

Many different kinds of bacteria, yeasts and molds are always present in the air, especially street air. The air of the country contains fewer bacteria than that of the city; there are fewer in high altitudes than in lowlands, and in mid-ocean there are practically none. About four times as many are found in shaded places as in sunlight. Apart from the local irritation of the respiratory tracts liable to be caused by the bacteria in the air, many infections as colds, grippe, et al, are common. Fruit exposed on the street vender's stand, especially such as peaches and fruits with rough skins, are covered with bacteria. Bacteria, unaided, do not rise in the air, but when mixed with dust particles which are moved by currents of air, are moved from place to place, so that the more dust, the more bacteria. More bacteria are found in summer air than in winter air. Flemming, by means of baloons, has found viable bacteria as high as 4,000 meters. The kinds of organisms vary in different localities; but in nearly all cases Bacillus subtilis or its spores, penicillum glaucum (blue green mold), pigment producing yeasts (red yeast), sarcina lutea (yellow chromogen producer), and various other forms are found. The tubercle bacillus, pyogenic cocci, and many others are commonly found in street air.

ANALYSIS OF AIR

(Recommended by the Committee of the American Public Health Association.)

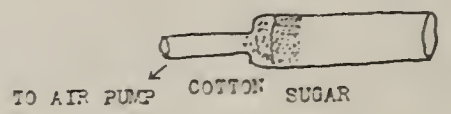
Use a glass tube of 15 mm. in diameter and 70 mm. long, with a smaller tube 6 mm. in diameter and 4 mm. long fused in one end. A plug of cotton is placed in the shoulder where the tubes join. On the cotton, a layer of sand which has been passed through a 100 mesh but will not pass through a 200 mesh sieve, is placed 10 mm. deep. The opposite end of the tube is plugged with a cork through which a glass tube is passed, measuring 6 mm. in diameter and 40 mm. long and bent at a 45 degree angle so as to prevent a direct precipitation of dust particles into the larger filter tube. The whole apparatus is sterilized. By means of suction, 5 cubic feet of air are drawn through the apparatus. The sand is then shaken into 10 mils of sterile water, shaken, and plated in petri dishes on ordinary nutrient medium. Incubate and count the colonies.



RUEHLE FILTER FOR COLLECTING MICRO-ORGANISMS FROM THE AIR.

SEDGWICK-TUCKER METHOD

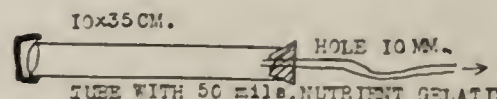
Sugar is used instead of sand.



SEDGWICK-TUCKER METHOD OF COLLECTING MICRO-ORGANISMS FROM THE AIR.

HESS METHOD

Gelatin medium is rolled inside the tube, cooled air drawn through the tube by suction. The tube incubated and the colonies counted.



HESS METHOD FOR COLLECTING MICRO-ORGANISMS FROM THE AIR.

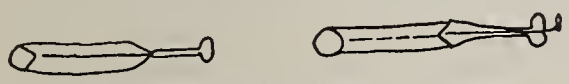
SOIL ANALYSIS

The upper 6 inch layer of the soil contains more bacteria than the lower layers, and few are found below 1½ to 2 meters. But this depends upon the presence of organic matter, moisture, et al. There are more bacteria in manured soil than in dry sandy soil. A test of uncultivated sandy loamy soil was made; it numbered over 100,000 colonies to the gram. A garden soil which had been cultivated for a number of years yielded 1,650,000 colonies and sewage-contaminated soil gave a count of 130,000,000 colonies.

Among the most commonly found bacteria were aerobic spore-forming bacteria as Bacillus subtilis, Bacillus proteus, those of the colon group and many pathogenic bacteria. Spores of anthrax live for many years in the ground, and Bacillus edematis, Bacillus typhosus and many others are soil inhabiters. The supply of bacteria is constantly being renewed by excretions of animals, from the air or water and by the continual process of fermentation, putrefaction and dissolution.

The analysis of earths is not satisfactory or accurate. For taking samples of the deeper layers a bore must be used.

A sample of earth is collected by means of a sterile borer



BORER FOR TAKING SAMPLES OF EARTH.

or spatula and placed in a sterile bottle. Certain amounts are weighed on covered sterile watch glasses and mixed with melted nutrient mediums—incubated and the colonies counted. Or the soil may be washed with sterile water, and portions of the water are then plated. In 1 gram of soil Lohnis found 3,750,000 peptone-decomposing colonies, 50,000 urea-decomposing colonies, 50,000 dentrifying colonies; 7,500 nitrifying colonies and 25 nitrogen-fixing colonies.

ISOLATION OF THE NITRIFYING BACTERIA

A sample of soil from a field which has been used for growing leguminous crops, as clover, is inoculated into the culture medium composed of ammonium sulphate 1 gram, potassium phosphate 1 gram, water 1000 mils and basic magnesium carbonate in excess. All organic matter must be absent from the soil or the bacteria will not develop. The inoculated medium is incubated and subcultures made and by the plating-out process pure cultures are obtained. These organisms will not grow well on ordinary culture media.

Each plant has a different organism and will not answer so well for alfalfa, or peanuts, etc. The cultures are placed on the market in liquid or dry form.

Some of the recognized nitrifying bacteria are the Azotobacter agile, Azotobacter chrococcum, Clostridium and Radiobacter. These organisms are of varying motility and include a number of species, appearing in various forms from oval bodies to bacillus-like forms. Other bacteria will also under certain conditions produce nitrogen as Bacillus pyocyaneus, prodigiosus, astrosporus and danicus which last is a spore forming aerobe. Aspergillus niger and penicillium glaucum may also issimilate nitrogen.

Bacillus ellenbachiensis, Bacillus Californiensis and the root-nodule forming bacteria Rhizobium mutabile.

A number of different cultures have been placed on the market, several of which have been patented. For leguminous plants several species of R. mutabile are sold, each species of plants such as beans, peas, clover, etc., having cultures which have been isolated from the root-nodules of their respective plants and have had their nitrifying powers increasd by passages through a number of cultures. Nitragin is the trade name of such a culture. Alinit consists of cultures of Bacillus ellenbachiensis, which is closely allied to B. megatherium and B. subtilis; this product is in the market in the form of a yellowish-gray substance and is made by mixing cultures of the spore-bearing bacillus with a base of albumen and starch. The cultures are grown in medias devoid of nitrogenous matter and in this way increasing their nodule producing power by making the bacteria, nitrogen hungry. These cultures and many other cultures produced in an experimental way have been generally successful for the soil production of nitrogen.

Nodule-forming bacteria as the Bacillus or Rhizobium radicola, which appear as actively motile, non-sporing aerobic long rods, grow on nutrient medium as non-liquefying small

colonies and appearing as small fat droplets.

A field of red clover may add more than 200 pounds of nitrogen to the acre.

Dentrification in fields may occur through certain chemical processes and through Bacillus coli, typhosus, fluorescens, pyocyaneus and others. This takes place only where there is an abundance of nitrates, changing the nitrates into free nitrogen, nitrates and gaseous oxides of nitrogen. When large quantities of fresh manure are added to a soil rich in nitrates or if manure and nitrates are added at the same time, dentrification is likely to occur.

Thoroughly wash the roots of the legume, as bean, clover, etc., under the tap. Select several large well developed and firm nodules, cut off and immerse for 3 minutes in a 1:500 mercuric chloride solution, then place in alcohol for 1 minute, remove from the alcohol with sterile forceps and immediately flame and then place the nodules on a sterile surface. Open the nodules with a sterile knife and inoculate some of the inner contents containing the bacteria into a petri dish containing a few drops of sterile water. Transfer 2 or more loops to a second petri dish containing a few drops of water and continue the dilutions until 4 have been made. Pour mannit agar solution into each dish. Distribute and incubate at 28 degrees C., for 6 to 8 days. The colonies appear as raised moist surfaces, rounded and entire, at first glistening but later change to an opaque white and vary in size from 1½ to 4 mm. in diameter.

ISOLATION AND GROWTH FROM LEGUME SEEDS

Wash the seeds in mercuric chloride for 3 minutes as previously directed. From this solution transfer to sterile water and thoroughly wash.

Drop several of the seeds into a large test tube containing the soft mannit agar or a loose mass of filter paper pulp. In case of larger legumes as soay beans, larger vessels must be used.

Inoculate the seeds with a 48 hour pure culture. Prepare a water suspension and from this take 1 mil for each tube. Nodules appear in from 10 to 20 days. Cultures should be developed in 4 to 6 weeks.

NITRIFICATION TEST

Place 20 mils of the nitrite formation solution in a series of 5 flasks, each holding 150 mils, inoculate with the cultures and test at 4 day intervals for the presence of nitrates and ammonia. The cultures should be incubated at 28 degrees C.

ISOLATION OF AZOBACTER

Inoculate 4 flasks of 100 mil capacity containing 20 mils each of the mannit solution with 1 to 2 Gms. soil, incubate at 20 degrees C., and every 2 days examine the films in hanging drop. When the culture has sufficiently grown, make dilutions in water blanks containing water 100 mils and clean sterile sand 50 Gms. (The sand is to break up the gelatinous clumps of azobacter.) Shake and transfer 1 mil to a second water blank, shake and transfer 1 mil to a third water blank. From the third dilution make petri dishes. Azobacter may be hard to isolate on account of the contaminating bacteria Bacillus radiobacter.

CULTURES FOR LEGUME INOCULATIONS

Inoculate mannit agar and also mannit solution with pure cultures. Use flat sided 500 mil flasks. In the liquid cultures use 300 mils of the media and in the agar cultures use 100 mils. Dilute a young culture with 5 mils sterile water, shake and inoculate 1 mil portions. Have the inoculations cover the whole surface. Incubate at 28 degrees C., for 5 to 10 days. Count the number of bacteria, bottle and label as to the number, kinds and date.

PLANT FOOD WITHOUT NITROGEN

Calcium sulphate 10 Gms., water 2 mils, mix, then add the solution composed of Potassium phosphate 4.6 Gms., Magnesium sulphate 2.3 Gms. and water 7 mils, after mixing add water sufficient to make 100 mils. This food is to be added when needed. In some cases several drops of chloride or phosphate of iron solutions are beneficial.

MANNIT SOLUTION

Mannit 15 Gms., Magnesium sulphate 0.2 Gms., Potassium phosphate 0.2 Gms., Calcium sulphate 0.1 Gm., Calcium carbonate 5 Gms., and distilled water sufficient to make 1,000 mils. The phosphate should be dissolved separately in a little water and the solution should be neutral to phenolphthalein with normal NaOH, then add the other ingredients. If solid medium is desired add to each L., 15 Gms. agar.

SOLUTION FOR NITRITE FORMATION

Ammonium sulphate, diabasic potassium phosphate aa. 1 Gm., Magnesium sulphate 0.5 Gms., Sodium chloride 2 Gms., Ferrous sulphate 0.4 Gms., Magnesium carbonate in excess (about 5 Gms.) and distilled water 1,000 mils. Sterilize the ammonium sulphate separately so as to prevent the loss of ammonia, then add to the other ingredients of the formula.

SILICATE JELLY FOR NITRIFYING BACTERIA

Add to 100 mils of an 8% solution of sodium silicate, 120 mils of HCl of such strength that 1 mil of the acid will neutralize 1 mil of the silicate. Tube and sterilize. If after sterilizing the media becomes too solid, it indicates an insufficient amount of the acid. Pour the silica acid mixture into petri dishes and then add the nutrient solution and neutralize with sodium carbonate solution and add a few drops in excess. After plates harden they are ready for inoculation. The nitrifying bacteria will live for some time on this media.

WASHED AGAR MEDIA

Heat the agar in distilled water until in solution, place in flasks and change the water several times in two weeks so that all organic substances are removed. Add precipitated calcium carbonate and hydrogen ammonium sodium phosphate and sterilize.

THE ORGANS OF THE URINARY SYSTEM

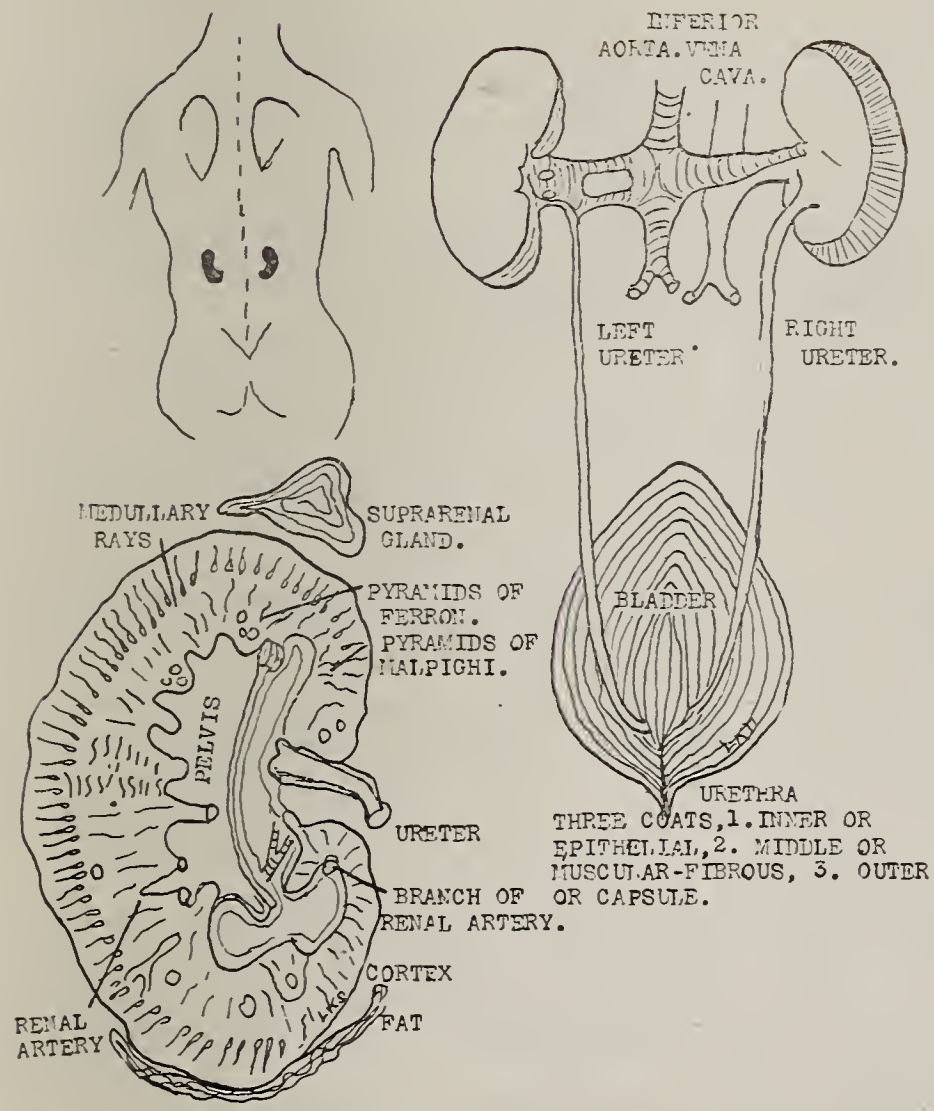
Consists of two kidneys, two ureters, one bladder and one urethral canal. The kidneys measure about 10 cm. long and weigh about 75 grams. The left kidney is the larger and is longer and narrower than the right kidney, and weighs less in the female than in the male. The vessels enter at the hilum and are the renal vin and artery and the ureter, which conveys the urine to the bladder, and from the bladder the urine is voided by the urethral canal.

The kidney is covered with a fiberous coat called the tunica adiposa, or the capsule, which encloses the incomplete layer of muscular fibre. The cortical substance forms the greater part of the kidney or gland (the kidney is a true gland) and is soft granular and contains the minute red globular bodies, the Malpighian bodies. Its substances is made up of the uriniferous tubules, capillaries, lymphatics and nerves held together by the intermediate parenchymatous tissue.

The medullary substances are pale reddish conical masses, called the pyramids of Malpighi, numbering 12 to 18 and their apices point towards the hollow space or sinus or pelvis. The medullary substance is firmer than the cortical layer and is striated. From the case of each pyramid medullary rays pass through the cortex and this portion is called the labyrinth of the cortex. The sinus is lined with mucus epithelial cells and these form a cup like cavity around each end of the pyramids and are called the calyx, which receive the urine from the tubules.

Each kidney has a renal artery which comes off from the aorta at right angles, and these supply the blood. After separation of the urine and the blood the latter returns by the renal or emulgent vein to the inferior vena cava. The nerve supply comes from the renal plexus.

The Malpighian bodies consist of a rounded tuft of capillaries and furnish the water portion to the urine, and the saline portion is furnished by Bowman's capsule. The convoluted tubules separate from the blood the various organic constituents as urea, uric acid creatimine, etc.



THREE COATS, 1. INNER OR EPITHELIAL, 2. MIDDLE OR MUSCULAR-FIBROUS, 3. OUTER OR CAPSULE.

SECTION OF KIDNEY.

ORGANS OF THE URINARY SYSTEM.

THE URINE

The urine is the fluid separated from the blood by the kidneys and is the principal means of removing the worn-out tissues, the nitrogenous and saline matters from the system. It is a clear bright amber-colored fluid with a bitter saline taste and characteristic aromatic odor when freshly discharged.

Every chemical and physical test should be supplemented by a thorough microscopical examination.

The entire quantity of urine for 24 hours should be examined or it may be divided into three parts, that taken on arising, after the mid-day meal, and before retiring. Urine may be preserved by arresting decomposition for some time by adding a small crystal of thymol or camphor to the specimen.—Phenol, chloroform and formaldehyde may interfere with chemical and bacteriological tests.

Women after 40 years of age usually lead a less active life than men, hence there is a decreased amount of urine, and at 60 years of age, there is a decrease of 60%. A healthy adult man will void about 1½ liters of urine in 24 hours; the amount varies and depends upon the amount of fluid or solids which have been taken. For 50 pounds body weight there is voided about 32 grams solids in 24 hours; for 100 pounds weight 57 grams solids; and for 150 pounds, 76 grams; and for 200 pounds weigh 88 grams of solids. About ½ of the solid constituents is urea.

The urine may be placed in conical sedimentation glasses or centrifuged. If it is thick and mucilaginous, a small amount of pancreatin and sodium bicarbonate may be added and the urine kept in a warm place for several hours and the turbidity if caused by urea, uric acid or urates will disappear on heating and reappear on cooling.

The total amount voided in 24 hours should be noted, the color, odor, reaction and sediment should be examined. The less water drank, the darker the color, and the more solid matter. The appearance is often an indication of the contents as:

Turbidity may indicate mucus or pus.

Milky indicates fat in suspension.

Much sediment may be pus, blood or phosphates according to the color.

Yellow to orange color may be caused by santonin, chrysophanic acid, etc.

Orange color indicates urobilin.

Greenish yellow to orange brown with yellow foam is caused by bile.

Green brown to brown black may be caused by phenol, guaiacol, napthalene, hydrochinon, resorcin and drugs of like nature. More color is seen after the urine has stood some time.

Red to brown tints may be caused by blood, rhubarb or senna.

Pink color is due to blood or pyramidon.

Red color is caused by hematoporphyrin, santonin, etc.

Reddish to brown black indicates blood.

Green color after indigo production, the yellow of the urine combining with the blue of indigo.

Blue color rarely caused by anything else than methylene blue.

Sediments in acid urine are usually composed of uric acid, amorphous urates, calcium oxalate, monoclinic phosphates, hippuric acid, calcium sulphate, cystin, leucin, tyrosin, xanthin, soaps of lime and magnesium, bilirubin and fat.

EXAMINATION FOR CRYSTALS

Sediments obtained by the sediment glass or centrifuge are placed on a clean slide, covered with a cover glass and the shapes of the crystals noted.

URIC ACID

Occurs in crystals shaped like a whetstone, single or rosettes, and composed of narrow slabs, of a yellow or brownish color. It is one of the largest crystals found in the urine and forms the brick dust sediment. They are dissolved by heat or alkalies and are increased by drugs of the caffeine group or Scilla and cause an increase in the gravity but not the volume of urine.

SODIUM AND POTASSIUM URATES

Appear as amorphous, colored, fine granules, often obscured in the microscopic field. They dissolve on heating and with oxalic acid form a precipate.

Ammonium Urate is seen as spherical amorphous brown crystals set with spicules or in delicate needle-like arrangements. It is soluble in acids separating out as uric acid.

Calcium Carbonate occurs as minute granules, single or in masses, and gives off carbon dioxide with acids.

Calcium Oxalate.—Occurs as envelope, octahedral, dumbbell shaped, colorless, highly refractive, non-striated crystals, often in forms composed of 2 or more bunches of needle-shaped crystals. They are usually associated with casts.

They are not affected by alkalies or acetic acid, but are dissolved by HCl.

Calcium Phosphate is rarely seen, occurring only in neutral or alkaline urine and as colorless needle-shaped or elongated prisms or tablets. It is soluble in acetic or other acids and insoluble in ammonia.

Primary Calcium Phosphate occurs rarely except with uric acid in acid urines. The crystals resemble somewhat those of uric acid, but are colorless and readily soluble in acetic acid.

Phosphaturia is a condition indicated as a milky pinkish deposit in the urine. The phosphates are from the food or from the breaking up of nuclein or proteins of the tissues.

Triple Phosphates of Ammonium, Magnesium and Calcium are rarely found in feebly acid urines but common to alkaline urines. The are large rhombic prisms of "coffin lid" shape; some of the smaller crystals may be mistaken for calcium oxalate, but the solubility in acetic acid prevents this error. In alkaline urines they may also appear as a variety of star-shaped or snow-flake like crystals with four or more arms with feathery borders.

Hippuric acid appears as long monoclinic prisms joined in various shapes.

Leucin occurs in sphere forms of various sizes closely resembling the appearance of fats, but they are insoluble in ether and of a brownish color with concentric striations and fine radiating lines.

Creatinin resembles the crystals of zinc chloride.

Tyrosin occurs in fine needles, grouped or bunched, crossing each other. They are insoluble in acetic acid but soluble in ammonia or HCl.

Xanthin resembles somewhat the colorless uric acid crystals.

Soaps of Lime and Magnesium resemble fatty acid or tyrosin crystals but do not give the tyrosin reactions.

Creatinin is rarely found in adult's urine. There is normally about 1 gram in children's urine. If found in more than 5 grams per 1000 mils of urine daily, death will result.

Bilirubin occurs as yellow or red rhombic plates, soluble in alkalies or chloroform, but not in ether. It will give the bile reactions.

Hematoidin is rarely found and is hard to distinguish from bilirubin. It is found in diseases of the bladder or kidneys which are accompanied with bleeding.

Indigo appears as delicate blue needles or amorphous

granules, usually found in urine which has undergone decomposition.

Cystin appears as colorless hexagonal plates which are soluble in HCl or ammonia but not in acetic acid, alcohol or ether, and will burn without melting with a green flame.

Cholesterin is rarely found. It appears as flat colorless plates with ragged edges; one corner of the plate appears as if it was punched out.

THE FORMED ELEMENTS.—These are the most important from the microscopical standpoint and are:

Epithelial Cells. Each different shaped epithelial cell is of diagnostic importance, for from the shape, the location from which they have come is determined. They may be rounded nucleated cells from the uriniferous tubules, deeper pelvis or lower genito-urinary tract, or they may be caudate or conical, or flat, or pavement form; these are large polygonal cells with a large nucleus, et al.

Leukocytes are found normally in small numbers.

Erythrocytes are also found normally in small numbers, but a large number is an indication of pathological conditions.

Casts are the most important morphological structures found in urine and may be of various kinds as:

Hyaline casts are secretions of mucus formed by the glomerules and which have remained in the tubule until they have become formed and shaped, taking the shape of the tubule which often becomes dilated before the cast is expelled. They probably come from the Henle loops. Albumen passing through the glomerule may coagulate and form hyaline casts and in certain pathological conditions and in acute infections of the epithelial cells of the tubules, a hyaline degeneration may occur, forming hyaline casts. Hyaline and their allies—cylindroids, are formed in the same substances and are found in concentrated and acid urines; as low specific gravity and neutral or alkaline urines cause an irritation and a sluggish flow of urine in some parts of the kidney. The more free the flow, the shorter the time the mucus remains in the tubules. Hyaline, epithelial and fine granular casts may be found in centrifuged urine of healthy persons. Hyaline casts repeatedly found in urine of persons over 40 years of age along with albumen, indicates a chronic interstital nephritis; but they may appear with severe physical strain, mental anxiety, or any disease which disturbs the metabolism of the circulation as acute infections. They appear under the microscope as colorless, very pale, transparent homogeneous parallel-sided, rounded or broken-

ended tubes. If bile is present in the urine they may be faintly colored yellow. They stain with Lugol's solution, picric acid, or fuchin solutions. When examined under the oil immersion lens the structure is seen to be not homogeneous but somewhat finely granular.

Epithelial casts are similar to the hyaline casts, except that the cast is filled or covered with epithelial cell from the tubules. The form of the epithelial cells indicates the region from which the casts originated. Irritation or passive congestion or tubular nephritis is indicated by the presence of these casts.

Blood casts resemble epithelial casts, except that the epithelial cells are replaced by blood cells, and they indicate a congestion, or hemorrhage of the kidneys.

Many epithelial, fine granular and hyaline casts show both white and red blood cells. Blood casts are frequently found in acute nephritis. When found in hematuria they indicate a bleeding from the kidney structure; and when found with uremia the prognosis is unfavorable.

Granular casts are degenerated epithelial casts and may be finely granular or coarse granular. When diffuse nephritis and degeneration occur in the convoluted tubule epithelial cells, the debris is thrown into the lumen of the tubules and after remaining there for some time causes the production of these casts which at first are with fine and later with coarse granules. If there is a large amount of urine passed, the debris will not remain long enough to form casts but may be seen in centrifuged urine as epithelial and granular debris; these debris are also seen after long and rapidly centrifuged urine, the casts being broken down.

Fatty or oily casts represent a fatty degeneration of the epithelial casts. They may be composed of fat or oil, or may be epithelial or granular casts containing oil globules due to an infiltration or degeneration of the cell contents. They indicate a serious condition and occur with chronic nephritis of any type.

Waxy casts, or amyloid casts are similar to hyaline casts, but show the amyloid reaction and are of a higher refraction, of a yellow-grey color and usually not attacked by acetic acid, and give the amyloid reaction of a mahogany color with Lugol's solution and then if sulphuric acid is added, turn a dirty violet color. They are rarely found whole, usually occurring in fragments. They are bent, straight or twisted and resemble molds from the convoluted kidneys. Their presence indicates a severe nephritis which

may be due to degenerated blood coagulum or an infiltration or degeneration of the kidneys.

Bacterial casts are casts filled with bacteria, indicating an infected condition.

Cylindroids resemble tube casts; they are cylindrical in shape, colorless and less refractive, longer, striated and often twisted, and consist of mucus. They are shorter and broader than hyaline casts.

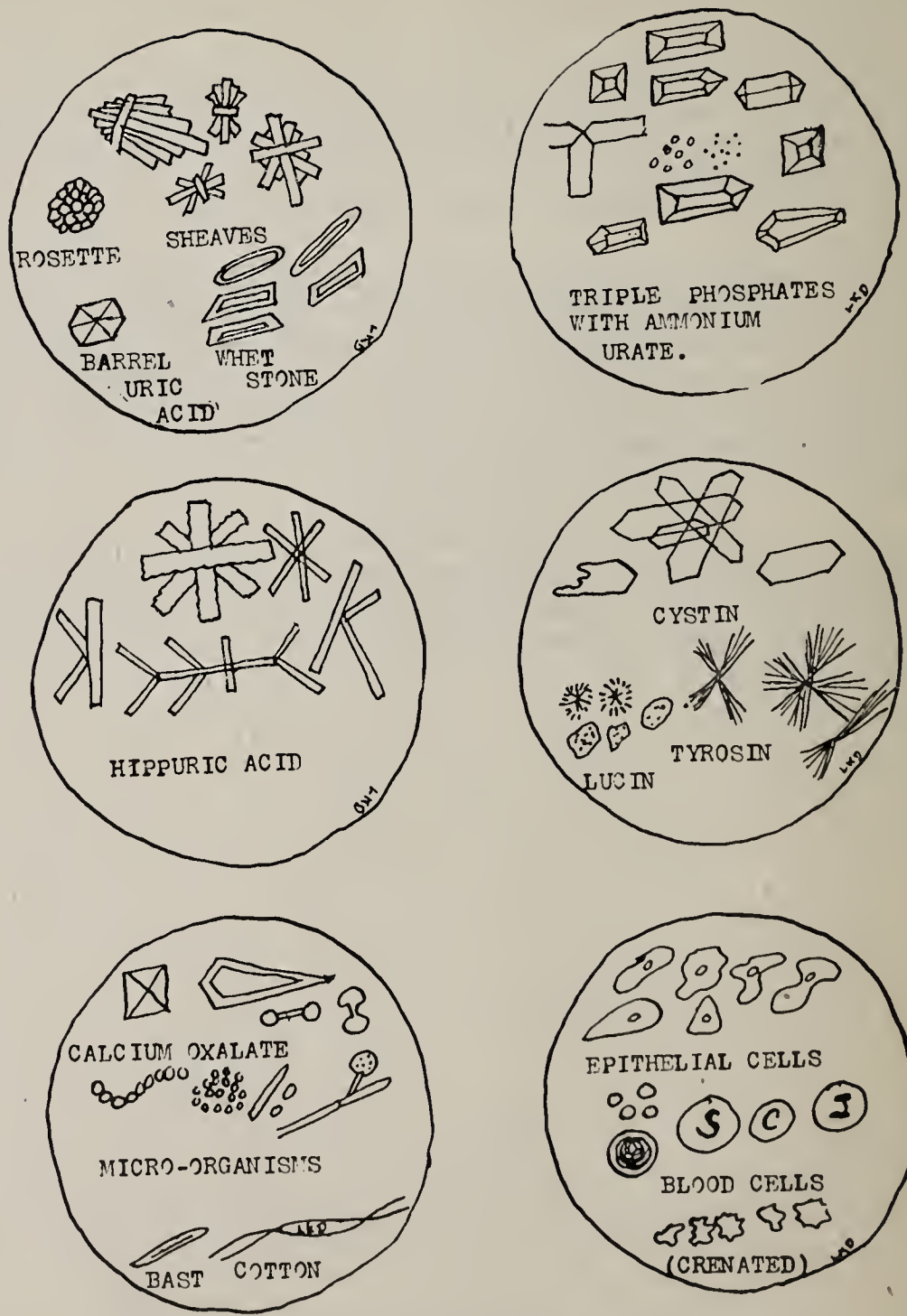
Tumor particles are from certain bladder and kidney diseases.

Spermatozea are long, cylindrical, tapering to a point at one end, and with a large, round, somewhat pointed head at the other end, and of a homogeneous structure, derived from the spremátic canals and are accompanied by testicular casts, prostatic plugs and large rounded cells with distinct nuclei.

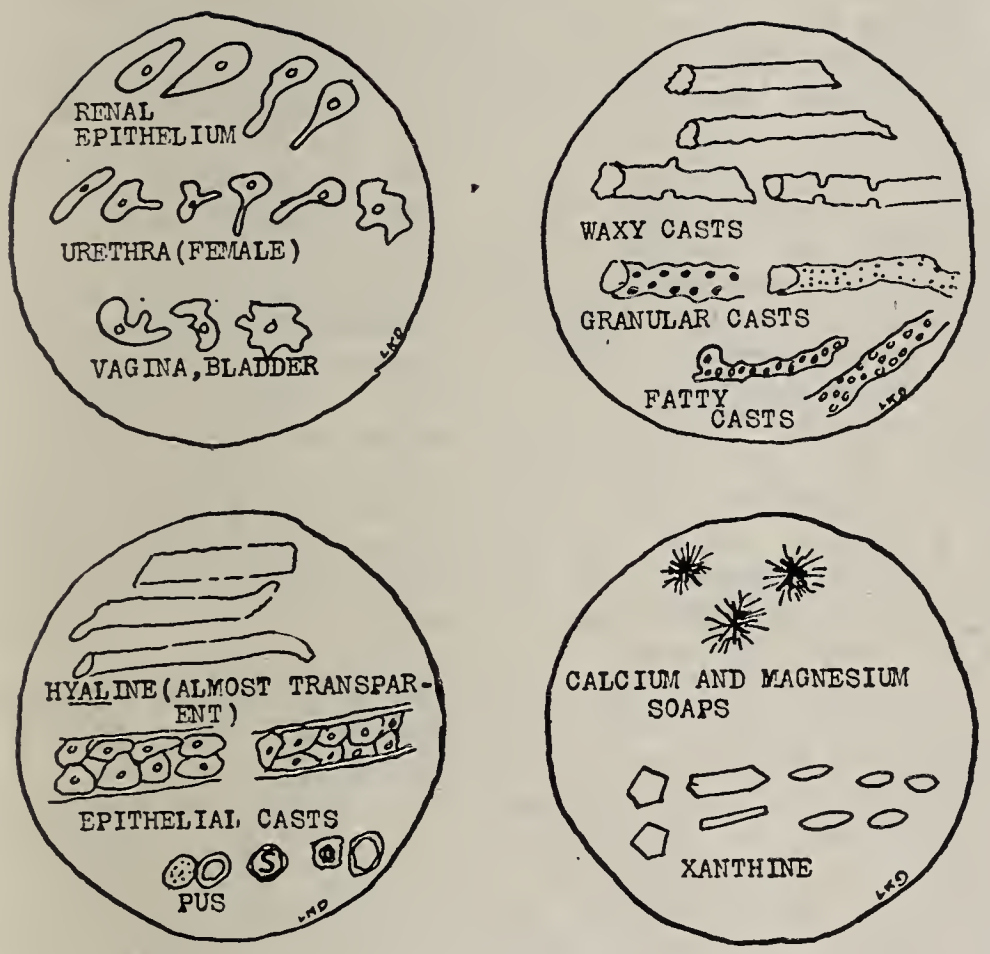
Hairs, fibres and other debris are found in urine, these coming from external contaminations.

EXERCISE

Place a small drop of centrifuged urine on a slide, cover and examine. Permit another specimen to air dry and stain as directed under Urine Stain as given on the pages of stain formulaes.



ELEMENTS AND CRYSTALS IN URINE.



ELEMENTS AND CRYSTALS IN URINE.

Feces are the solid excrements, the matters which an animal ejects from the lower end of the intestinal tract, and consist in the greater part of those portions of food which on passing through the ailmentary canal have been rejected as comparatively worthless in the office of nutrition. They consist, in the higher animals, of about 75% water and 25% organic remains of the foods.

The normal stool is twice daily and varies from 100 to 200 grams and when dried about 45 grams. It is increased by a rich vegetable and starch diet and decreased by a diet rich in animal proteins. Prolonged constipation may cause the intestines to retain as much as 20 kg. of fecal matter. The consistence and form of the stool depend upon the amount of water present,—soft with a pure vegetable diet (about 80% water) and harder with an animal diet rich in proteins (60% water), and with a mixed diet about 75% water. The color is due to uroilin, hence on standing the surface oxidizes and becomes darker. Different substances may change the color, as huckleberries—a blackish; cocoa—grayish; iron, manganese and bismuth—dark brown or black (due to sulphides); calomel—greenish (biliverdin); santonin, rhubarb or senna—yellowish; blood—scarlet red to black (hematin); bile—a golden yellow to green; biliary obstruction—grey to white; and the greater the number of movements the lighter the color. Ammoniacol decomposition is due to micrococcus urea and other bacteria.

The stool microscopically shows fruit seeds, as grape or cherry; fruit skins, woody vegetable fiber, undigested pieces of food, gall stones, mucus, worms, et al.

Stools from Fat Indgiestion are large, semi-solid gray, acid reaction, and often contain much mucus. Often they are loose, frothy, gray and very acid. Butryric acid odor is common. Fat occurs in small soft curds, or give an oily appearance to the stool (babies), the fat so occurs as fatty acids or soap microscopically.

Stools from Sugar Ingestion are loose, yellow, sometimes green, frothy and acid. The odor is that of acetic or lactic acid. These stools are very irritating to the skin. They often contain mucus. In diarrhea, microscopically they show unabsorbed fat and undigested food particles which have passed rapidly through the intestine.

Stools from Starch Ingestion are loose, yellow-brown, often frothy and acid. Usually with an acetic or lactic acid

odor, but if a change in the starch to fat, the odor is butyric. If very acid, the stools are very irritating to the skin. They contain large amounts of mucus and starch, the starch either unchanged or partly converted to dextrin.

Stools from Protein Indigestion are loose, bronish and alkaline. The odor is foul or musty and seldom contain mucus.

Stools from Ingestion with Fermentation are the same as the other types with the addition of those due to fermentation. Usually frothy, contain mucus and the acidity or alkalinity of the reaction is increased, according to the type of fermentation present, and the odor is more acid or putrefactive.

The stool must be rubbed to a homogeneously fluid condition; if it is solid, it must be rubbed with sterile water; or if too fluid must be concentrated by sedimentation or centrifuging, when it will appear in distinct concentric layers; the lower layer will contain the larger crystals, muscle fibers and the heavier vegetable cells as wood, stone or bast cells. The surface layer will contain the fats and fatty acids in crystal form and the lighter cellulose cells. The first process is to centrifuge with HCl, then with absolute alcohol, then with ether—this will remove the alkaline salts, the real oils, chlorophyll and fats, leaving only the undigested residue and the acid salts. The residue of meat depends upon the amount of meat eaten and the muscle fragments appear as irregular polygonal or rounded and striated or homogeneous. If the striations are distinct it shows poor digestion for meats, but if homogeneous with the striations indistinct and the fragment yellowish in color, the meat digesting power is good.

Connective tissues. On addition of acetic acid, smells and becomes homogeneous.

Casein occurs in lumps and is deeply stained by the bile pigments a brownish color eternally, the interior remaining whitish. It is dissolved with 5% KOH solutions.

Fats. Neutral fats occur in droplets or masses with irregular rounded corners, colorless and with a high melting point. Fats occur in irregular masses more or less stained with bile pigments. They are insoluble in water but soluble in chloroform. When feces are rubbed with 50% alcohol and stained with soudan red the fat appears as golden droplets. Fat in crystal form occurs as delicate needle-like crystals.

Soaps are amorphous masses or crystals. They may be colorless or stained with bile pigments, or they may appear as colorless needle-like crystals arranged like sheaves of wheat; and the crystals are shorter and broader than those of fatty acids.

Calcium salts are insoluble in hot water, alcohol or ether. They may appear as crystals of magnesium and calcium salts.

Mucus. When the stool is mixed with water and permitted to trickle down a blackened plate of glass, it appears as homogeneous faintly marked coutour masses.

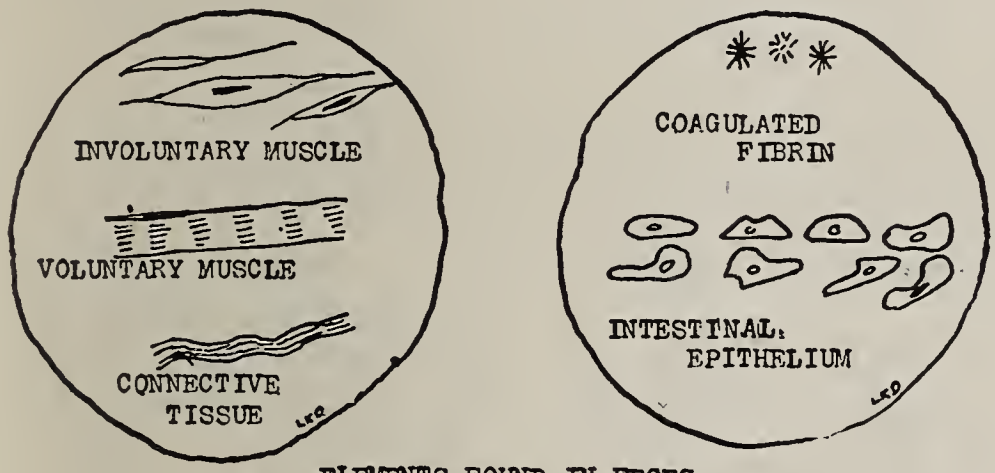
Blood cells are present in small numbers; large numbers indicate pathological conditions. The red cells from the lower colon are much distorted and hard to recognize.

Epithelial cells are constantly found. The shape is diagnostic of their source.

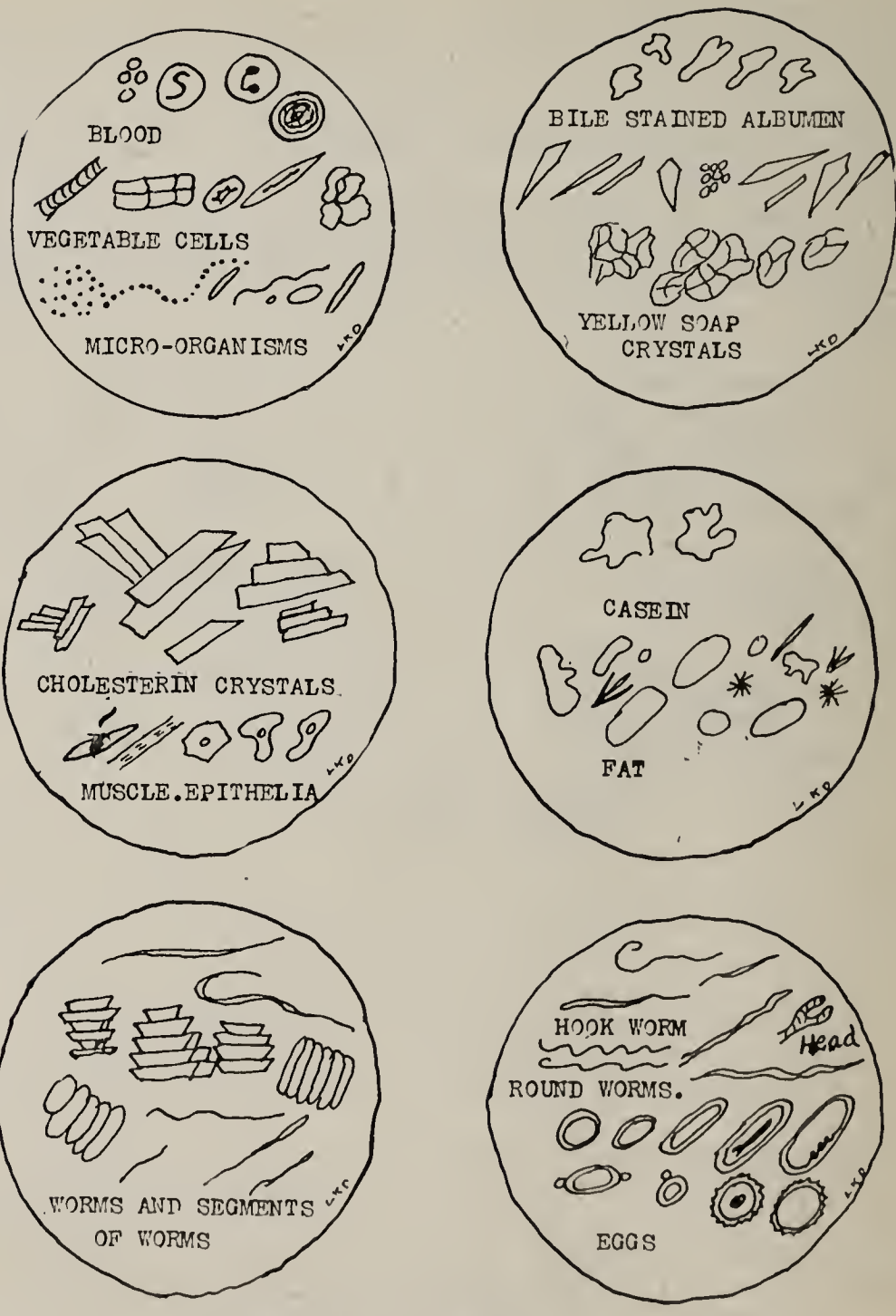
Pills, tablets, etc., may pass through the intestinal tract without being dissolved,—many of them show but little dissolution.

Cholesterin appears as gall-stone like masses, which are soluble in alcohol, and when evaporated on a slide show the typical crystals.

One gram of normal dried stool shows: Total fat solids, 225 mgm., or 22.5%; fatty acids, 86 mgm., or 37.9%; total soaps, 74.7 mgm., or 33.4%; total neutral fat 64.4 mgm., or 28.5%.



ELEMENTS FOUND IN FECES.



ELEMENTS FOUND IN FECES.

EXAMINATION FOR WORMS AND EGGS IN FECES

The specimen to be examined is fished with a large platinum loop, the drop placed on a slide and covered with a cover glass. The low power is sufficient to distinguish the ova and parasites.

For amebic dysentery the stool must be kept warm until examined, and examined on a warm stage, or by warming the slide from time to time over the flame.

If it is desired to stain the slide it may be stained with aniline gentian violet solution, when everything but the ova will be stained a violet.

If the ova are not visible on examination, about 20 grams of the feces are passed through a 32 thread silk sieve, diluted with saline solution and 10% formaldehyde equal parts, and centrifuged 1 minute at 1800 revolutions. The sediment is treated with 1 mil of the solution containing 12 grams citric acid, 2 mils formaldehyde and 86 mils water; it is then agitated for 2 minutes and again centrifuged for 30 seconds, stirred with a clean pipette and again centrifuged for 30 seconds; on examination the sediment will contain only the protozoa and ova.

Bass Method. The feces are mixed with 10 volumes of water, filtered through gauze, centrifuged and washed twice with saline solution and after each washing centrifuged. Calcium chloride solution of the gravity of 1.250 is added and the eggs will float to the top where they may be skimmed off; to make sure all the eggs have been found, the mixture is diluted with water until the gravity is 1.050, again centrifuged, and the sediment examined for eggs.

EGGS AND WORMS FOUND IN FAECES.
TRICHURIS TRICHURA.
ASCARIS CORNIS
AGCHYLOSTOMA DUODENALIS
(HOOK WORM)
STRONGYLOIDES
STERCORALIS.
ASCARIS LUMBRICOIDES
N.AMERICANUS.
NEMATODE EGGS
F.DUMARQUAYL
(NATURAL SIZE)
OXYURIS VERMICULARIS
EGGS.
F.PERSTANS
ASCARIUS CANIS
(NATURAL SIZE).
EGG OF
DIPHYLLOBOTH-
RIUM LATUM.
F.LOA
(NATURAL SIZE)
TRICHINELLA SPIRILUS
F.BANDEROTT
(NATURAL SIZE)
DIPYLIDIUM CANINUM
DRACUNELIS MEDINENSIS
(NATURAL SIZE)
TAENIA SAGINATA
TREMATODE. NATURAL
SIZE.
FILARIIDAE.
TAENIA SOLIUM
EGG.

A BRIEF CLASSIFICATION OF SOME OF THE MOST IMPORTANT OVA

Cestodes.

1. With an operculum.
   Brown ova.
   - 41x58 microns .....Diplogonoporous grandus.
   - 41x50 microns .....Dibothriocephalus parvis.
   - 45x70 microns .....Dibothriocephalus latus.
2. Without operculum.
   a. With a single membrane.
      Ova spherical, thick, opaque.
      21x36 microns .....Taenia solium.
      Ova ovoid, thick, opaque.
      25x35 microns .....Taenia saginata.
   b. With 2 thin transparent membranes.
      30x40 microns .....Diplidium caninum.
   c. With 3 transparent membranes.
      Daviainea madagascariensis.
   d. With 3 transparent membranes, the third being the piriform apparatus.
      About 68 microns.....Hymenolepis.

Trematodes.

1. With an operculum.
   A. Small ova, less than 50 microns.
      a. Ovoid ova.
         - 16x25 microns .....Heterophyes heterophyes.
         - 11x29 .....Motorchis truncatus
         - 21x34 .....Opisthorchis noverca.
      b. Prominent operculum.
         1. No prolongation.
            11x30 microns .....Opisthorchis felineus.
         2. With a prolongation.
            16x30 .....Clonorchis sinensis.
      c. Ova bulging on one side.
         25x40 microns .....Dicroccelium lanceatum.
   B. Large ovoid ova measuring up to 170 microns.
      1. Ova containing no embryo.
         - 55x95 microns .....Paragonimus westermanni.
         - 53x100 microns .....Echinostomum ilocanum.
         - 75x125 microns .....Watsonius watsoni.
         - 72x125 microns .....Echinostomum malayanum.
         - 75x125 microns .....Fasciolopsis buski.
      2. Ova with embryo (but embryo not seen in fecal matter).

72x150 microns .....Gastrodiscus hominis.
80x150 microns .....Fasciolopsis rathouisi.
80x140 .....Fasciola hepatica.
84x170 .....Fasciola gigantica.

Nemathelminthes.

1. Nematodes.

A. Ova with smooth wall and a single envelope.

a. With a thick wall.

One side bulging, the other flat.
23x50 microns .....Oxyruis vermicularis.

Regular and ovoid.
39x57 microns .....Physaloptera caucasica.

Each hole with a clear plug.
25x55 microns .....Triocephalas trichuris.

b. Transparent.

1. With 2 to 4 blastomeres.
40x60 microns .....Ankylostomum duodenale.
40x60 microns .....Ternidens deminutus.
40x70 microns .....Necatur americanus.

2. With 4 to 8 blastomeres.
40x70 microns .....Esophagosatomum brumpti.

3. With 8 to 32 blastomeres.
45x80 microns .....Trichostrongylus probolurus
48x80 microns .....Hemochus contortus.
45x83 microns .....Trichostrongylus instabilis.
48x82 microns .....Trichostrongylus vitrinus.

4. Twice folded thick embryo.
32x54 microns .....Strongyloides intestinalis

B. Ornamented wall.

Mammillated, brown.
44x60 microns .....Ascaris lumbricoides.

Cribbed with depressions, yellow.
65x75 microns .....Ascaris canis.

Cribbed, depressions, none at pole, brown.
42x66 microns .....Eustrongylus visceralis.

2. Acanthocephalus.

Ova with 3 envelopes, embryo with spines in rows, and with lozenge shaped depressions.
48x85 microns .....Gigantorhynchus gigas.
50x100 microns....Gigantorhynchus moniliformis.

SOME OF THE MOST COMMON INTESTINAL PARASITES

1. Rhizopoda (Sarcodine).
   Entameba coli, causes some forms of dysentery.
   Entameba histolytica, causes liver abscesses and dysentery.
   Entameba gingivalis, one of the causes of pyorrhea.
2. Coccidiaria.
   Eimeria stiedae, infects epithelium of bile ducts and liver.
   Isospora gigemina, usually from intestines of dogs and cats.
3. Trematodes or Flukes.
   Fasciolidae hepatica—liver flukes caused by eating raw liver; rarely in man.
   Clonorchis endomicus, common in man's liver—pathogenic.
   Opistorchis felineus, infests the gall bladder and bile ducts.

   A. Intestinal flukes.
      Fasciolopsis, common, in dyspepsia and diarrhoea.
      Fasciolopsis ilocana.
   B. Liver flukes.
      Paragonimus westermanis, common, in coughs with rusty sputum.
   C. Blood flukes.
      Schistisomum hematobium, comomn, infests the circulatory system.
4. Cestodes or tape worms. Segmented ribbon-like worms.
   Tania saginata. 3 to 10 meters long, with a small pear-shaped head, appears 2 months after ingestion of the worm eggs.
   Tania solium, the pork tape worms, small, globular head with 26 to 28 hooklets, may invade organs as eye or brain in cyst form.
   Hymenolepsis nana—dwarf worm—the smallest of the tape worms, about 0.5 to 1.25 cm. long and 0.1 cm. thick. It has 4 suckers and 25 hooklets.
   Dibothriocephalus latus—Russian tape worm. It has an olive-shaped head, broad segments numbering 3,000 or more, each about 1.25 cm. wide—the mature worm is 10 or more meters long.
5. Nematodes or Round Worms.

Strongyloides stercoralis measures from 2.5 cm. to 30 cm. in length, an infestion causing diarrhoea.
Filaria mendinesis are thread-like and 50 to 75 cm. long.
Filaria perstans rarely are over 7 cm. long and are in the deep fatty tissues.

6. Trichocephalus trichiuris—Whip worm, about 5 cm. long.
Trichinella spirilus may be 15 cm. long and is encysted in the muscles.
Strongylidia.
a. Eustrongylus gigas, one of the largest worms, 0.8 cm. wide and 100 cm. long, found in the pelvis of the kidney.
b. Tridontophorus diminutus, very small worm with 3 teeth, and about 1.25 cm. long.

7. Agchylostoma duodenale—hook worm about 0.8 cm. long. Neactur Americanus—American hook worm, inhabitant of the lower intestines.

8. Ascaris.
Ascarius lumbricoides—eel worm, from 12.5 to 37 cm. long and 0.5 cm. in diameter, infests the upper intestines.
Oxyuris vermicularis—pin or seat worms, from 0.4 to 1.25 cm. long.

They must be as poisonous as possible to insects and as insoluble as possible to the plants—as arsenic, which is slowly soluble to the plant but rapidly soluble to the animal. Copper sulphate acts in the same way but is washed off the plant by the rain and dew. Enormous losses are caused yearly by plant fungi and parasites.

Animal parasites may be classified as:

1. Biting. Those which have jaws, and chew or devour the plant tissues as food, such as the cobbage worm and potato bug. Sprays are of little value as the poison must be placed on the parts to be eaten. Paris green, arsenate of lead, zinc salts and hellebore are the most frequently used. Paris green contains arsenic and copper and is destructive to animals and slightly to vegetable life.

2. Sucking. Those which do not consume plant tissues but insert a beak or sucker into the plant cells, extracting the juices. San Jose scale, Aphis and squash bug are examples. Applications to the plant surfaces are of little value. The poison must be placed on the parasite body, so that it will be killed by its caustic action or by smothering as in sprays of lime and sulphur solution, soap washes and tobacco extracts.

3. Hard-bodied, as beetles. Arsenite compounds must be placed so that they will be eaten. The larvae or caterpillars are easily destroyed by external application.

4. Slugs, snails, etc. Paris green, carbon disulphide, or arsenic and antimony compounds are effective.

5. Day feeding. A spray of arsenic compounds or emulsions of lamp oil or alkaline tobacco extract.

6. Night feeding. Are destroyed by arsenic powders sprinkled on the plant parts, or arsenic and antimony mixtures; salt and molasses smeared around the plant stems keep away slugs and snails.

7. Nesting insects. Formalin spray is used.

8. Webs and nests. Sulphur fumes; or sprinkle with sulphur and ignite by setting fire to paper or other inflammable material tied on a pole.

9. Shade tree borers. Thick white wash applied in the early spring, the infested parts cut out and burned. Or carbon disulphide injected into the galleries of the borers and the openings plugged with clay.

Plant parasites:

1. Slime molds, such as those producing club root on cabbage and the Cruciferae.

2. Bacteria cause about 130 known plant diseases, as pear blight, cankers, galls and black rot. The remedy is to cut out the infected part and burn it, then apply to the cut and surrounding parts bichloride 1 in 1,000. All seeds suspected of or exposed to the infection should be soaked in this solution before planting.

3. Fungi causes the largest number of plant diseases, as smut, rusts and mildew. Oat smut in 1913 caused $6,500,000 damage in the United States.

SOME OF THE MOST COMMONLY USED FORMULAS

Arsenate of calcium for soft-bodied insects feeding on leaves, lice, et al. Spray plants attacked with feeding insects, but not lettuce or cabbage, or the consumer may be poisoned. The arsenic is boiled with 1 to 2 parts of slaked lime and water, and made alkaline with lime water.

Arsenate of lead for beetles, hard-boiled insects, chewing beetles, caterpillars, etc. Arsenate of sodium 3 parts, lead acetate 7 parts, mixed with water and the precipitate collected and diluted with 40 parts of water, and used as a spray.

Or lead arsenate 3 pounds to 50 gallons of water, sprayed on the fruit trees after the flower petals fall; again spray in 2 weeks and again in July.

In order that the arsenic sprays be made to stick to the leaves, the solutions must be made alkaline with lime water.

London purple is an arsenic compound of lime; paris green is acetate-arsenite of copper. They are used dry or as a spray when mixed with lime water, or when mixed with a mixture of 1 part to 2 parts slaked lime and 2 parts rosin soap in the proportion of 2 tablespoonfuls to the gallon of water.

Kerosene emulsion. Crude rosin soap made by using 2 parts powdered rosin, 1 part sodium carbonate and 30 parts of water, and crude castor, linseed, cottonseed or whale oil to saponify. It is an efficient spray for aphides and sucking insects. Or it may be made by using ½ lb. hard soap mixed in 1 gallon boiling water, then 2 gallons of kerosene is added and it is thoroughly mixed until it becomes creamy. 1 part is diluted with 10 parts of water for foliage spraying, but for spring and winter spraying 1 part is mixed with 5 parts of water.

Salt or unslaked lime sprinkled over damp places inhabited by slugs and snails will destroy these parasites.

Powdered Insecticides, as Paris green, hellebore, pyrethrum or insect powder, tobacco, et al. For soft bodied in-

sects they are more effective when mixed 15 parts with 1 part of powdered rosin soap; this will cause the powder to adhere to the plants.

Volatile Insecticides, as Hydrocyanic acid gas are excellent but it is too dangerous for man to use. Crude sodium cyanide is much safer. It is placed in crocks, sulphuric acid is added which develops the gas; but great care must be taken as the fumes are poisonous to man. It is used in houses for the destruction of bed bugs, roaches, etc.

Carbon disulphide is less dangerous and is effective for ants, wasps, snails, moles, field mice, etc. It is slow but safe, and repeated applications give good results.

Copper salts are a specific poison for all lower forms of life.

Copper sulphate when used in the Bordeaux mixture is efficient for algae, water plants, molds, insects, mildew and rusts. It is composed of 15 grams copper sulphate to 4 liters of water; this is added to lime water in the proportion of 1 part to 3 parts of fresh slaked lime in 1,000 parts of water.

Copper carbonate may be used as a dusting powder, or in a spray mixed with oils or soaps.

Copper sulphate 1 pound and water 15 gallons is made as a stock solution. For use it is diluted 1 part with 7 parts of water.

Copper sulphate solution precipitated by ammonia water and redissolved in ammonia water is also very valuable as a spray.

Sulphur in the form of sulphur dioxide gas is valuable for mildew. The sulphur is burned over water, and under the covered plant.

Sulphur mixed with lime and water forms a good spray for mildew, smuts and rusts.

Formalin 1 in 100 parts of water sprayed or wiped on leaves or placed in dishes under the plant is efficient for the smaller insects.

Bordeaux mixture is made by placing 5 pounds of copper sulphate in a cloth sack suspended in 5 gallons of water. In another vessel lime is slaked in the proportion of 1 pound of lime to 1 gallon of water. When ready to use, mix equal parts and dilute as required.

Bordeaux mixture improved. Copper sulphate 1¼ lbs., lime water 17 gallons and water sufficient to make 100 gallons.

Ammoniacal copper solution is stronger than the Bordeaux mixture and is composed of copper sulphate 5 ounces, 26% ammonia water sufficient to precipitate and redissolve the precipitate, and 50 gallons of water.

Lime-sulphur wash, sulphur 10 pounds, stone lime 10 pounds and water 50 gallons.

Postassium sulphide 5 ounces and water 10 gallons.

Sulphuric acid 1 part and water 1,000 parts is efficient for rose mildew and similar fungi.

Corrosive sublimate 2 ounces and water 15 gallons is good for potato scab, etc. It must be kept in crocks or wooden vessels.

Nicotine or tobacco sprays are good for soft-bodied sucking insects as aphis on roses, sweet peas and other delicate plants. Tobacco stems are steeped for 3 hours in hot water in a closed vessel in the proportion of 1 pound of tobacco stems to 3 gallons of water. It is used as a spray.

Bacterial rat and mice exterminators have been on the market for sometime, such as ratins, rat virus, azoa, Danysz virus, etc. These consist of the Bacillus murisepticus and Bacillus typhimurius mixed with corn or oat meal or in a liquid form and are mixed or sprinkled on the rat food. While these preparations are non-pathogenic to man or other animals except rats and mice they are not as successful as it has been hoped they would be.

PHYSIOLOGICAL ASSAY OF DRUGS

Many drugs cannot be assayed by chemical methods so they must be tested as to potency on animals. This testing is better than chemical testing, for it will give the action of the drug on the animal as well as the strength.

THE DIGITALIS GROUP

The physiological or biochemical pharmacologist classes Digitalis as one of the heart stimulant group, which includes Apocynum, Convallaria and Epinephrine as well as the drugs classed by the United States Pharmacopoeia, Digitalis, Strophantus and Scilla. These drugs belonging to this group are assayed in the same way.

THE ASSAY

The U. S. P. directs the frog method of assay. This method is probably the one hour method originated by Famulener and Lyon and consists of determining the smallest fatal dose, M. L. D., or the smallest dose which will kill a frog in 1 hour. The Reed and Vanderkleed method is based on the M. L. D. which will kill in 24 hours.

Frogs are used and should be freshly caught. They must be healthy and carefully handled, kept in water and wet moss until they are placed into the storage tank with running water, or in a tank where the water is changed at least 4 times a day. The water should be at a temperature between 10 and 15 degrees C. These precautions must be taken or the frogs will die. All experiments must be carried out at the temperature of the water and never exceeding 20 degrees C. The frogs must be of the same species, of Rana viridis, or pipens and of the same weight, of about 15 to 20 grams and must have been kept at least 3 days before being used. When the frogs are to be used, they are each placed in small wide-meshed wire cages with a removable top. The cages are lowered into the large tank which should have the bottom covered with water. Since the dose is computed per gram body weight, frogs of any weight may be used, but those weighing about 20 grams give the best results, except in Strophanthus testing in which frogs of 10 to 17 grams are used and for Digitalis those of 18 to 21 grams. The weight of the frogs used in the same test should be within 0.1 gram, although in assaying the range limit may be from 0.5 to 3 grams.

THE SOLUTION TO BE TESTED

The solution to be tested should be so diluted that not more than a total of 0.5 mils liquid is given per gram weight

of frog and not more than a total of 0.5 mils should be injected. The fluid extract or tincture should be diluted with water or saline solution so that it contains not over 2% alcohol. If the solution contains more than this amount it must be carefully evaporated and normal saline added to make up the volume. It is essential to have the alcoholic content of the different solutions to be tested, of the same uniform strength. If any precipitate occurs, the solution must be thoroughly shaken before injecting.

THE INJECTION

The injection is made into the anterior lymph sac by means of the glass syringe or a graduated fine-pointed pipette graduated in 1/100 mils. The frog is wrapped in a towel, held on its back, the mouth forced open and the needle or pipette inserted under the tongue and through the floor of the mouth and into the lymph sac. The solution is injected by gravity, or gently blowing into the pipette, or by slight and gradual pressure on the syringe piston. Air must not enter the circulation, nor must the skin be punctured for in the frog as well as in all cold-blooded animals, the punctures do not readily close and hemorrhage ensues and will spoil the test. After injection return the frog to the cage and place the cage in the large tank.

THE PITHING

After exactly 1 hour after injecting, the frog is removed from the cage, wrapped in a towel, leaving only the head exposed, and holding the wrapped frog in the left hand, the nail of the right forefinger is passed lightly along the spine until the articulation between the skull and vertebral column is felt; this is the point where the cerebral-spinal canal has no bony covering. The canal is punctured at this point by inserting a narrow knife blade or cutting with scissors, the brain is destroyed by inserting a probe at this punctured point and pushing the probe into the brain cavity and moving the probe gently from side to side. The probe point is now turned and the spinal canal is destroyed in a like manner. This final stimulus of the nerve cells causes a discharge of motor impulses to the muscles of the body giving a series of convulsive twitchings or contractions which quickly cease and the body becomes toneless and relaxed and the reflexes abolished.

The frog is pinned to a frog board with the abdomen side up, the skin over the abdomen is pinched up and slit to the mouth, the abdomen wall is divided slightly over the

median line to avoid cutting the anterior abdominal vein. By a transverse cut the sternum is divided (the junction of the anterior abdominal vein with the heart is preserved), the chest girdle is then divided in the middle line, the inner blade of the scissors being kept hard against the sternum to avoid tuching the heart beneath. The divided halves of the pectoral girdle are pulled apart and the heart is seen enclosed in a thin membrane,—the pericardium. Pick up the pericardium with the forceps and slit it open. On the posterior side of the heart is a slender band of connective tissue—the fraenum—which must be divided.

THE TEST

The ventricle should be in systolic standstill; that is, if the heart is completely paralyzed the dose has been excessive; and if the dose has been insufficient the heart will continue to beat. Basing the doses on these results, other series of frogs are injected with progressively increasing or decreasing doses as the case may be, still further diminishing the dose until the minimum dose of the drug per gram weight of the frog has been ascertained, WHICH WILL CAUSE A PARALYSIS OF THE HEART ACTION IN ONE HOUR AND WHEN STIMULATED BY TOUCHING THE HEART WITH THE END OF A PROBE, THE HEART WILL RESPOND WITH ONE ADDITIONAL CONTRACTION. If the heart will not thus respond the dose has been excessive. The test must be repeated if remains of the drug are found in the lymph sac unabsorbed; or if any one frog exhibits decreased or increased susceptibility to the drug. After obtaining the approximate strength of the drug, inject a second series of frogs, using dose limits much narrower than those previously used. The test should be carried to the third or fourth series and then should be compared with the dose of the standard preparation.

Guinea pigs may be used as outlined under the Aconite Group. A more accurate method is: Fasten the frog to a frog board; after pithing, the heart is exposed and a small sized bent pin attached to a silk thread is hooked into the heart tip and the silk thread is attached to a heart lever, the lever counterpoised and the heart action recorded on a kymograph drum. Three stages are recorded; the normal tracings are started on the revolving drum for a space of about 6 cm. then a toxic dose of the drug is injected and the tracing continued until the heart stops, which will be in 15 to 20 minutes. In the last stage the rhythm of the heart is

changed and the extent of the contractions and relaxations of the ventricle and auricle undergo certain modifications; the rhythm is slower; the inhibitory apparatus is set into activity and the slowing is due to the prolongation of the pause in distole. The ventricles contract to a smaller size, that is they have emptied themselves more completely than under normal conditions. In normal conditions the ventricles do not completely emtpy themselves. The increased contraction is due to the excessive inhibitory activity on the cardiac muscles. The capillary muscles undergo the same changes contracting more strongly and more completely. The second is due to an excessive inhibitory activity while the cardiac action is less developed. The rhythm of the ventricle and consequently on the pulse is very slow and irregular. During diastole the ventricle dilates more completely while the systole varies in strength. The third stage is complete inhibition, or complete stoppage, of the heart action.

THE STANDARD

Th standard is obtained by comparing the solution to be tested with that of ouabain. It is figured as:

Standard ouabain dose is to the found unknown dose as the standard dose of the drug is to the dose of the unknown strength drug.

The standard M. L. D. of ouabain is 0.0000005 per gram weight of frog and for Digitalis is:

Tincture 0.006
Fluid extract 0.0006
Fresh tincture 0.0006

For Strophanthus:

Tincture 0.000006
Fresh tincture 0.00006

For Scilla:

Fluid extract 0.00006
Tincture 0.0006

For Apocynum:

Fluid extract 0.00016

For Convallaria:

Fluid extract 0.0003

THE HEART TONIC UNIT or the H. T. U. is 10 times the M. L. D. per Gram weight of frog, when tested under normal conditions. The following formula is used in calculating the number of H. T. U. from the results of the test:

1 divided by 10 times the M. L. D. of the sample, multiplied by the result obtained by dividing the standard M. L. D. by the average standard M. L. D. will equal the number of heart units per Gram or mil.

Example:

M. L. D. of the Tr. Digitalis equals 0.02.

M. L. D. of the standard Tr. Digitalis equals 0.012.

Average standard of the M. L. D. of Tri Digitalis U. S. P. equals 0.006.

Using the above formula for the just stated data:

$$\frac{1}{10 \times 0.02} \times \frac{0.012}{0.006} \text{ equals } \frac{0.012}{0.0012} \text{ or 10 H. T. U.}$$

The Gold Fish Method consists in placing a certain number of gold fish in a measured amount of water, to which has been added a known amount of a known strength solution of the drug to be tested, noting the time required

to kill the fish. This is the control. Then the same process is repeated, using instead of the known strength solution, the solution of the drug of unknown strength, comparing the time for killing, the strength of the unknown solution is determined. The water temperature should be that of the water in which the gold fish are usually kept. This method is very accurate and can be performed by the Pharacist.

THE ACONITE OR HEART DEPRESSING GROUP

The U. S. P. gives an Assay for Aconite; it should also give under this heading of heart depressants, Gelesmium and Veratrum. These drugs are assayed by the same method.

THE ANIMAL. Guinea pigs of from 250 to 350 grams weight are used and require no special preparation, except that they be in good health, the hair clipped or shaved from the abdomen for a space of about 2 cm., and the denuded surface painted with Tr. Iodine just before the injection.

THE DRUG. Tincture, fluid extract, or extract may be used. If the tinctures are used the alcohol must first be evaporated as directed in the Digitalis assay. If the solid extract is to be tested, or the powdered drug, these must first be dissolved in a suitable quantity of menstrum to render the dose accurately measurable. In all cases the preparation should be so adjusted so that the dosage should range from not less than 0.5 to not more than 2 mils in quantity.

THE ASSAY. Inject the drug by means of a syringe or graduated glass pipette, by inserting through the shaved skin and injecting subcutaneously in the abdominal wall, using in a series of pigs the following doses: 9-10, 10-10, 11-10, and 12-10 of the standard dose of the preparation to be standardized, for each 250 gram weight of pig, gently massaging the injected liquid away from the point of injection before withdrawing the needle. The animals must be so marked that the dosage will not be confused; this is best done by placing one or more stain colors on the backs of the pigs. The pigs are placed in cages and examined at the end of 12 hours (24 hours more reliable) and a record made of the living and dead pigs. This is a preliminary test in which a wide range of doses were used and will determinate the approximate strength of the preparation examined. Basing the doses, on these results, other series of pigs are injected with increasing or decreasing doses as the case may be, until the M. L. D. is determined. This dosage is then checked by injecting 4 pigs, 2 with the smallest dose found to kill and the other 2 with the largest dose which did not kill, thus determining the true M. L. D.

The following are the standard M. L. D. per gram weight guinea pig:

Aconite:

Fluid extract	0.00004
Tincture	0.0004

Extract	0.000004
Veratrum:	
Fluid extract	0.0002
Tincture	0.002
Extract	0.00006
Gelsemium:	
Fluid extract	0.0015
Tincture	0.015
Extracts	0.0004

THE PHYSIOLOICAL ASSAY OF CANNABIS

THE ANIMAL. Take a young dog of one or two years of age, full grown, short haired and from 8 to 12 kilos in weight and of a known susceptibility, which has previously been determined by administering the test dose of 0.01 gram per kilo weight. The operator must be perfectly familiar with the behavior of the dog. This is acquired by having the dog under observation, and noting the peculiarities and behavior under normal conditions and with the best housing and feeding. This is very important, otherwise the operator will not be able to distinguish the symptoms of inco-ordination. The same dog may be used for a number of tests, providing the tests are made at least 3 days apart. It is best to permit a week to pass between the tests, in order that the dog may completely recover from the effects of the previous dose. It is not advisable to use the same dog for more than 6 months, as the dog will become accustomed to the test and lie down before the drug acts and thus render the test unreliable. The same operator who has been obsering the dogs must carry out the tests.

PREPARING THE DOG. Several dogs with which the operator has become familiar and which are of proven susceptibility are weighed and all food except water is withheld for 24 hours (12 hours is sufficient). The dog is placed in a room free from all outside disturbances; he must not see or hear other dogs and after the administration of the dose should be disturbed as little as possible.

ADMINISTRATING THE DOSE. The drug in the form of extract, or fluid extract of known strength is placed in a hard gelatin capsule or formed into bread pills. The dog is held between the sitting operator's knees and his head is held by placing the thumb and index finger of the left hand between the jaws and just back of the teeth, thus forcing the mouth to remain open. The dose is placed on the back of the tongue, the mouth closed and held closed by the right hand; by stroking the throat with the left hand, the dog is readily made to swallow the medicine. A small amount of water is then given the dog.

THE CONTROL. The U. S. P. standard preparation of 0.01 gram per kilo weight is given to a second dog of proven susceptibility.

THE ASSAY. The symptoms are divided into 3 stages: The first stage is one of excitement; the second inco-ordination; and the third drowsiness. A short time after administering the dose the dog vomits and then becomes excitable,

and in 1 to 2 hours inco-ordination occurs, beginning with the loss of leg and head control, so that when the dog is standing, the feet are placed wide apart to maintain balance and when nothing occurs to attract the attention of the dog, the head drops, the body sways from side to side and when the dog attempts to walk, it staggers and falls. If the dog is sharply spoken to, it momentarily recovers and the typical effects disappear, but return in a few moments to the incoordination. As the action of the drug progresses the muscular incoordination becomes greater and the dog passes into the third stage, which at first exhibits depression; then lassitude increases until finally the dog sinks to the floor exhausted and passes into a deep undisturbed sleep. These effects usually disappear in several hours. The INCO-ORDINATION or the reaction to be observed, is the end reaction, that is the smallest dose which will produce muscular inco-ordination in 1 hour. The dogs must be under constant observation during the entire test up until the stage of untroubled sleep.

THE STANDARIZATION. A number of dogs, usually 4, are used, 2 of which are given doses of 10-10 and 11-10 of the standard dose of the preparation to be tested for each kilo weight. The other 2 are given doses of the standard preparation; these are used as the controls. If the preliminary tests show the drug to be above or below the standard, other dogs are given progressively smaller, or larger doses until the smallest dose per kilo is determined, which will produce inco-ordination in 1 hour. The relative strength of the drug is computed between the minimum and the standard minimum dose which will produce inco-ordination, or ataxia, in walking, drooping of the head, gentle swaying of the body, in 1 hour. On the third day, the standard dose and the test dose are reversed in administration to the dogs; that is the drug to be tested is given to the dogs which had received the known standard drug, and the known standard drug is given to the dogs which have received the unknown strength drug, 3 days before.

The standard doses which will produce inco-ordination in 1 hour, per kilo weight:

Fluid extractum Cannabis 0.03
Extractum Cannabis 0.004
Tinctura Cannabis 0.3

Cannabis may also be tested by using frogs. The standard for which is 6.2 mgms. for each 20 grams weight of frog. This dose should deeply narcotize or kill the frogs.

THE ASSAY OF ERGOTA

Ergota may be tested in the same way as Pituitary solutions and also by the cock-comb method.

THE STANDARD. The cock-comb method consists of determining the minimum amount of ergot necessary to cause the same degree of bluing in the cock-comb or wattles, as that produced by a given amount of the standard, when introduced into the crop or injected intramuscularly.

THE APPARATUS. Accurately graduated syringe and scales.

THE ANIMAL. Normal 12 to 18 months old, thoroughbred leghorn roosters of as nearly the same size, weight and age as possible. They should weigh from 1200 to 1600 grams.

PREPARATION. All food withheld for 24 hours.

INJECTIONS are made deep into breast muscles, or the drug introduced into the crop by means of a soft rubber tube.

THE ASSAY. One cock is injected with a dose of the standard preparation; another is injected with the same dose of the preparation to be tested and after 1 hour the color of the two combs is compared. The maximum cyanosis occurs in this time; the color soon begins to fade and at the end of 2 hours, depending upon the dose, the color of the comb is nearly normal again. From these two doses the dose of the unknown strength drug is decreased or increased until the amount is found which will give approximately the same intensity of color as that shown by the standard solution. The same roosters may be used several times, but at least 3 days must pass before the test is again made. After an approximate dose is obtained, it is verified by other tests on other roosters. This method gives only relative results.

The ergot standards for raising blood pressure:

Fluid extractum Ergotae 30 mm. caused by 0.08 mils per kilo weight of dog.

Cornutol 12 mm. caused by 0.04 mils per kilo weight of dog.

Ergotina 8 mm. caused by 0.04 mils per kilo weight of dog.

The alkaloid ergotoxine causes the bluing of the comb. The alkaloid tryamine raises the blood pressure and acts on the uterus. The alkaloid cornutine also raises the blood pressure. The Crawford method of testing consists of injecting 5 mils of the fluid extract; the Edmunds and Hale method consists of injecting 1 mil of the fluid extract deep

into the breast muscles which should cause a blackening of the comb in 1 hour; this equals 2½ mgm. ergotoxine phosphate, which is their standard preparation used for comparison. They use white leghorn roosters of less than 1 year old. The Hatcher method uses frogs; Blumefield uses cats; and the standard is that amount which will stop the cat's heart in 90 minutes. Kehrer used the isolated cat uterus muscles; and Goodall has a standard of 20 minims injection, which should raise the blood pressure 2 mm. of mercury in a 1500 gram animal.

THE ASSAY OF PITUITARY PREPARATIONS

The analysis for determining the activity of these preparations resembles that of the Suprarenal gland and Adrenalin, and depends on the fact that consecutive doses or injections of a certain quantity of the active constituents under rigidly defined conditions increases the blood pressure on anesthetized dogs to the same degree.

THE ANIMAL. Dogs must be healthy and maintain a good normal blood pressure and of about 10 kilos in weight, completely anesthetized, until all reflexes are abolished, and must be sensitive to changes of 10% in the amount of pituitary preparations injected and must be sufficiently sensitive that 1 and not over 2 mils of the test solution will cause a rise in blood pressure of at least 1 cm., or 0.05 mils raise the blood pressure not over 24 mm. (12 mm. by the U tube). If the pressure is raised 40 mm. or over, the solution is too strong and must be diluted. The injections must be at least 15 minutes apart.

THE PREPARATION OF THE DOG. The dog should be completely anesthetized by injecting 0.3 grams of trichlor-tertiary butyl alcohol in oil, per kilo weight of dog, and after complete anethesia the carotid artery and femoral veins are exposed as directed in the assay of the Suprarenal gland and the artery connected with the manometer and kymograph in the same way.

THE SOLUTIONS. A standard solution of a 1 in 20,000,000 beta-iminazolyl-ethylamine-hydrochloride is prepared; the test solution of the dried powdered gland 0.1 gram gland in 100 mils of water is made up and decanted or filtered, and 1 mil of this solution should equal the standard dose.

THE ASSAY. 1 mil of the standard test solution is injected into the vein, the rise of blood pressure recorded on the kymograph, and the injection repeated in 15 minutes. If the rises are not equal repeat the injection in another 15 minutes. When the injections of the unknown by increasing or decreasing the amount of the injections causes the same rise in the blood pressure, then by altering the injections of the standard and test solutions until the rise is the same with definite amounts of each, the strength of the test or unknown strength solution is computed. This method is not as accurate as desired, hence the

ISOLATED UTERUS ASSAY

This consists of the very delicate contractility of the uterus muscles of a virgin guinea pig or cat, suspended in Loch's solution, one end of the muscle fastened to the bottom of the container and the other end fastened to a movable heart lever recording on a kymograph drum, the solution to be tested causing the muscle contractions.

THE ANIMAL. The uterus is taken from a young virgin guinea pig weighing from 250 to 350 grams, and which was killed by a blow on the head. A strip measuring about 1 to 2 cm. long is taken from one horn of this uterus, which must be normally slender and without congestion.

THE APPARATUS. A kymograph is used with a writing lever magnifying from 3 to 5 times also a Locke's constant temperature bath (38 degrees C.) and an inner container for the muscle in solution, and arranged so that a constant stream of oxygen may bubble through the solution.

THE ASSAY. One end of the muscle is fastened to the bottom of the container; the other end is attached to the heart lever by a silk thread—the heart lever being adjusted to write on the smoked kymograph drum; the container filled with Locke's solution; a stream of oxygen is permitted to bubble through the solution. Tracings are started, and then a definite quantity of standard solution mixed with Locke's solution is injected into the solution around the muscle fiber, and the contractions are recorded. The Locke's solution is drawn off through a suitable opening in the bottom of the container; the muscles washed and the container filled with fresh Locke's solution. This is tested; if contractions are recorded the muscle must again be washed with Locke's solution until no contractions are recorded; then introduce the test solution into the container and let the muscle contractions be recorded. When the maximum contractions have been obtained and no further rise is shown, the final quantity is secured by injecting alternately the standard and the solution to be tested, washing the muscle between the tests and replacing the Locke's solution, until the average amplitude of the curves produced by several consecutive injections are practically equal. The assay should be started by using 0.01 mil of the standard. If a dose of 0.5 mils will not produce contractions, the muscle should be discarded and replaced by another. The Locke's solution must be kept at 38 degrees C., and a continuous stream of oxygen must bubble through it.

THE SUPRAENAL GLAND ASSAY

This assay depends upon the characteristic transitory quantitative rise in blood pressure in dogs, produced by intravenous injections of properly diluted submaximal doses in aqueous solutions, and comparing the solution strength to be tested with a solution of known strength of laevo-methyl-amino-ethanol-catechol.

THE APPARATUS. There are needed a large kymograph, manometer, accurately graduated all glass syringes, several small sharp pointed scissors, grooved director, hemostats, glass seekers, several dog clamps, small glass cannulas, silk ligatures and a dog board.

THE ANIMAL. Dogs, cats or rabbits. The U. S. P. prescribes a dog of medium size and in good condition and weighing from 8 to 14 kilos.

PREPARATION. The dog is anesthetized completely. Any anesthetic will serve which will not depress the heart; several are in use. The following will suffice: 1-100 gram morphine sulphate is injected subcutaneously, per kilo weight; 45 minutes later 2 grams acetone-chloroform per kilo is given per os, 1.6 grams for dogs weighing from 5 to 7 kilos, and 2 grams for those weighing 10 to 12 kilos. The acetone-chloroform is shaken with 4 mils of alcohol until dissolved; then 4 mils of water is added, shaken and filtered; it is then ready for injecting. Or the dog is tied, and to avoid being bitten, the operator should draw the hind leg back and inject trichlor-tertiary-butyl-alcohol into the peritoneal cavity. The solution is prepared by dissolving the weighed amount in as small a quantity of alcohol as possible and bringing the solution to the required amount by adding water and just sufficient alcohol to keep the drug in solution. The drug is injected in the amount of 0.04 gram per kilo weight of the dog. Or, a general anesthetic may be used and after the dog is completely anesthetized a canula connected with an ether bottle may be inserted in the tracheae. Within 5 minutes after injection, the dog becomes restless and some muscular inco-ordination takes place and soon the dog is prostrated and in about 20 minutes complete anesthesia is obtained. If the animal is anesthetized by ether of the injection methods, it will remain in this condition for 21 to 24 hours and at the end of the test it must be killed. If it is desired to have the animal recover after being given the injections, an injection of morphine is first given and the dose of trichlor-tertiary-butyl-alcohol is then

0.2 grams. These methods are accomplished often with nausea and affect the action of the heart very little.

After all voluntary movements have ceased (if all movements have not ceased, the U. S. P. prescribes sufficient curare) and atropine sulphate .01 gm. and .002 gm., respectively, per kilo body weight to be given intravenously and proved by electrical stimulation then the animal is placed on its back on the animal board, the feet tied, the hair clipped from the throat and an incision is made exposing the carotid artery and the saphenous vein. Ether may be used; cannula is inserted in the trachea and the cannula connected to the ether bottle. A cannula is tied in the artery and vein or as the U. S. P. directs, a cannula is tied in both femoral veins. The incision is covered with gauze moistened with normal saline solution, and the animal left undisturbed for from 1½ to 2 hours to permit a part of the anesthetic to pass off. The gauze is removed and a cannula tied to both femoral veins; each cannula is fitted with a short piece of rubber tubing for connecting to the syringes and manometer; or the carotid artery leading towards the brain is tied off, and the part leading towards the heart is also tied off with bull dog clamps and a cannula inserted between the clamps and connected with a bottle filled with magnesium sulphate solution, or with a ½ normal sodium carbonate solution (these solutions are to prevent the blood from clotting and also to prevent the blood from entering the manometer tubes) and the other end connected with the manometer by means of a glass Y. It is always best when using the injection method of anesthesia to open the tracheae and insert a cannula so that the animal may receive artificial respiration if necessary.

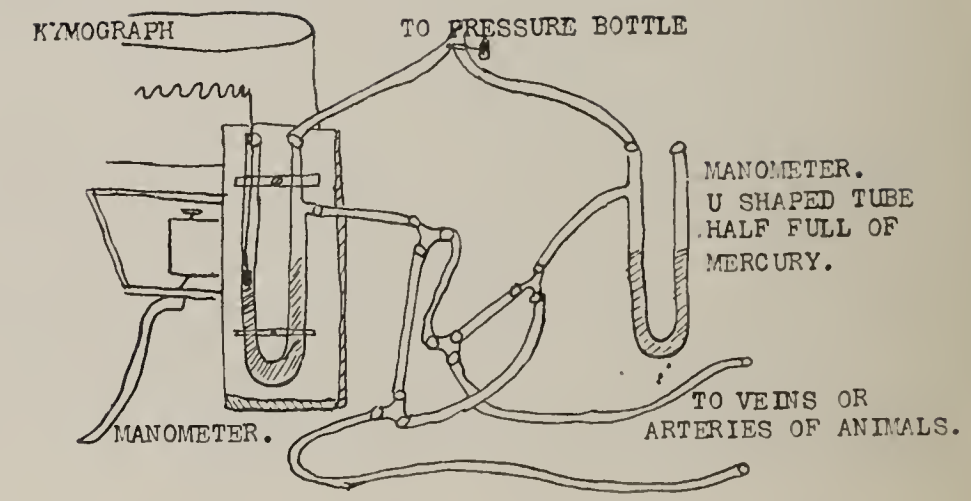
THE STANDARD SOLUTION. This is a 1 in 10,000 solution of laevo-methylene-ethanol-catechol in normal saline solution; and the solution to be tested consists of 1 gram of finely powdered gland in 100 mils of water containing 10 mils dilute hydrochloric acid and macerated 24 hours, with frequent shaking, then filtered.

THE INJECTIONS are made into the jugular or femoral veins through the cannula. The vein is best, on account of being farther from the heart.

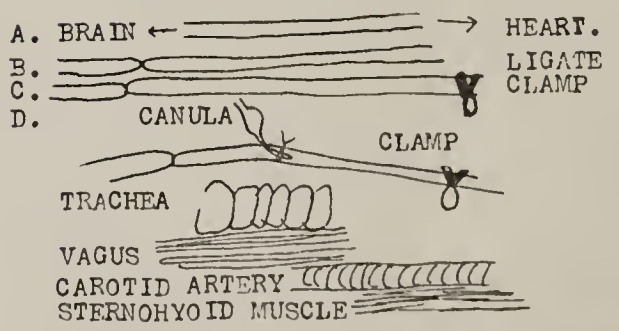
THE ASSAY. This consists in comparing the normal kymograph tracings showing the rise in blood pressure caused by injecting the standard solution, with the tracings caused by the unknown solutions. A normal tracing of about 8 cm. is taken, showing the normal blood pressure

then by means of the glass syringe, 0.05 mils of the standard test solution is injected, or as directed by the U. S. P. 0.15 mils, per kilo weight of the dog; this should cause a rise in blood pressure of 30 to 60 mm. If this pressure exceeds this, injections should be made not later than 5 minutes, or not sooner than 3 minutes, of 0.5 mils, and so on until a satisfactory dose is obtained. Then 1 mil of the preparation to be tested is injected. The injections must be made at the same rate and only after the tracings have been normal for at least 3 minutes. Then inject increasing or decreasing doses of the test solution until the desired pressure is obtained; then alternate the injections of the standard and the unknown strength preparations and the pressure of both remaining the same and showing uniformity in the tracings, the strength is calculated and expressed in terms indicating the test solution activity compared with that of the standard. Always remember to permit sufficient time to elapse between the injections for the pressure to return to normal and that the blood pressure readings are twice the actual on account of the two sides of the U tube of mercury multiplying.

THE STANDARD. 1 gram dried gland should equal 10 mgm. laevo-methylamine-ethanol-catechol.

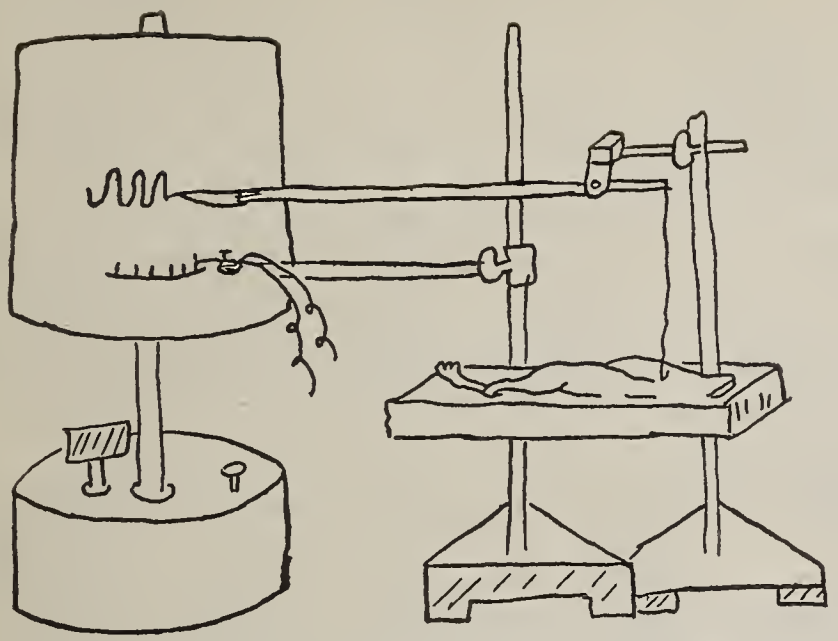


BLOOD PRESSURE ASSAY.

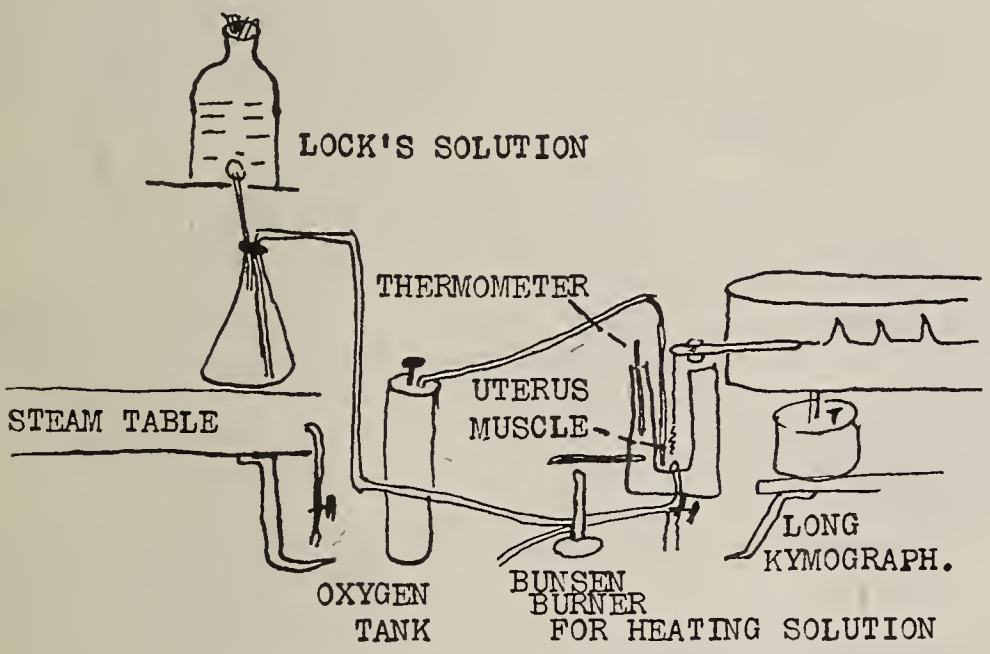


CONNECTING BLOOD VESSELS AND MANOMETER.

INJECTING FROG.



KYMOGRAPH RECORDING FROG HEART ACTION.



Group	Animal	Dose	Result
1. DIGITALIS.			
Tr. Digitalis	Frog	0.006 per gm.	Kill in 1 hr.
Fidext	Frog	0.0006 per gm.	Kill in 1 hr.
Tr. Fidext (from dry drug)	Frog	0.0006 per gm.	Kill in 1 hr.
Tr. Strophanthus	Frog	0.00006 per gm.	Kill in 1 hr.
Tr. Strophanthus (of seeds)	Frog	0.000006 per gm.	Kill in 1 hr.
Fidext Squill	Frog	0.0006 per gm.	Kill in 1 hr.
Tr. Squill	Frog	0.006 per gm.	Kill in 1 hr.
Fidext Apocynum	Frog	0.00016 per gm.	Kill in 1 hr.
Fidext Convallaria	Frog	0.0003 per gm.	Kill in 1 hr.
Ouabain	Frog	0.0000005 per gm.	Kill in 1 hr.
2. ACONITE.			
Fidext Aconite	Guinea pig	0.00004 per gm.	Kill in 24 hrs.
Tr. Aconite	Guinea pig	0.0004 per gm.	Kill in 24 hrs.
Ext. Aconite	Guinea pig	0.000004 per gm.	Kill in 24 hrs.
Fidext Veratrum	Guinea pig	0.0002 per gm.	Kill in 24 hrs.
Tr. Veratrum	Guinea pig	0.002 per gm.	Kill in 24 hrs.
Ext. Veratrum	Guinea pig	0.00006 per gm.	Kill in 24 hrs.
Fidext Gelsemium	Guinea pig	0.0015 per gm.	Kill in 24 hrs.
Tr. Gelsemium	Guinea pig	0.01 per gm.	Kill in 24 hrs.
Ext. Gelsemium	Guinea pig	0.0004 per gm.	Kill in 24 hrs.
3. CANNABIS.			
Fidext Cannabis	Dog	0.03 per Kilo.	Incoordination
Ext. Cannabis	Dog	0.004 per Kilo.	in 1 hour
Tr. Cannabis	Dog	0.3 per Kilo.	
4. BLOOD PRESSURE.			Raise pressure
Fidext Ergot	Dog	0.08 per Kilo	30 mm.
Cornutol	Dog	0.04 per Kilo	12 mm.
Ergotina	Dog	0.04 per Kilo	8 mm.
Laevo-methylamine-ethanol-catechol 1-100,000 sol.	Dog	0.15 per Kilo	30 to 60 mm.
Suprarenal Gland	Dog		Compare raise in pressure with Laevo-methylamine-ethanol-catechol solution.
Beta-iminazolyl-ethylamine hydrochloride 1-20,000 sol	Dog		Compare the rises in pressure.
Pituitary Liquor Hypophysis	Dog		

PREPARATION OF HUMAN TISSUES FOR MICROSCOPICAL EXAMINATION

Unless the tissue is to be examined soon after removal from the patient or following autopsy, it must be preserved. This may be accomplished in a number of ways; the two most common are cutting the characteristic portions in pieces about 2 to 3 cm. thick and preserving in 4% formaldehyde for 12 to 24 hours, or in Zenker's fluid (potassium bichromate 2.5 grams, corrosive sublimate 5 grams and water 100 mils, and just before use 5% glacial acetic acid is added) for 24 hours. In skin or mucous membrane lesions the plane of incision should be perpendicular to the surfaces. In tumors care must be taken to obtain portions of the adjacent lymph nodes, or any nodules which may be suspected of new growths. Specimens from bones or calcified areas must be fixed in 4% formaldehyde for 24 hours before decalcification. The specimens after fixation are washed in running water for 12 to 24 hours and placed in 80% alcohol until used.

There are three general methods of preparing sections after fixation—Freezing, Embedding in paraffin and Embedding in celloidin. The paraffin method gives thin sections and requires no special apparatus other than found in laboratories. The celloidin method is very much slower than the paraffin method and unless handled by one having had much experience is not satisfactory. The freezing method is the quickest of all and gives good results except with fatty and friable tissues.

THE PARAFFIN METHOD

The blocks of tissues are taken from the fixing or preserving material; the surfaces are cut parallel and placed in 80% alcohol for 2 to 4 hours; then transferred to 95% alcohol for 6 to 24 hours (according to the side of the piece); and finally to absolute alcohol for 6 to 24 hours; this is for the purpose of dehydration. The blocks are then immersed in chloroform for 6 to 24 hours, transferred to chloroform saturated with paraffin, at room temperature for 6 to 24 hours, and then placed in a paraffin bath at 55 degrees C. for 2 to 4 hours. This is done to eliminate all the chloroform. The blocks are removed from the paraffin bath and placed in paraffin which has been melted, and permitted to cool until a thin pellicle forms; after the blocks are placed in this melted paraffin, it is cooled as rapidly as possible by placing the container in a dish filled with water. The walls of the con-

tainer in which the tissue is placed should be rubbed with glycerine so that the paraffin embedded tissue is easily removed, and a strip of paper bearing the number of the tissue should be imbedded with it for recognition. The blocks after cooling are trimmed with a knife until they have a border not less than 3 mm. around the imbedded tissue. A wooden or fiber block is warmed over the flame and the paraffin block is placed on the warm surface, so that sufficient paraffin will be melted to stick the tissue to the block, and the block is numbered with the number of the tissue specimen. In cutting sections they should adhere by the edges so that a ribbon will be formed. These ribbons are laid on the surface of a dish containing water at 44 degrees C. The surface of a slide is painted with Mayer's glycerin-albumen (equal parts white of eggs and glycerin, and 1% sodium salicylate added to prevent decomposition). The sections are floated on this slide, dried in the air and placed in the incubator at 55 degrees C. for 2 to 12 hours. The paraffin is removed by passing the specimens on the slide through several changes of xylol, followed by 95% alcohol.

THE CELLOIDIN METHOD

The trimmed blocks of tissue are placed in 95% alcohol for 6 to 24 hours for dehydration; they are then placed in "thin celloidin" (4% celloidin in equal volumes of 95% alcohol and ether) for several days, with occasional shakings; and for several days in "thick celloidin" (8% celloidin in equal volumes of 95% alcohol and ether). At the end of this time they are removed with forceps and placed on blocks, fresh thick celloidin being poured over them and dried in the air. They are then dropped in 80% alcohol to harden. If large sections are desired, the blocks are taken from the thick celloidin, placed face downward in a tray folded from paper and containing thick celloidin, and permitted to dry, protected from dust. The drying takes 6 to 8 hours, when the blocks will have contracted to a firm horny mass; they are transferred to 80% alcohol. The drying process must be carefully watched, for too much drying will spoil the preparation. After passing through the alcohol, the blocks are trimmed as directed for the paraffin blocks and cemented to the wooden or fiber blocks with a drop of thick celloidin; after drying in the air for several minutes they are placed in 80% alcohol until used.

THE FREEZING METHOD

The blocks may be frozen with ether, ethyl chloride spray or liquid carbon dioxide. The specimens are frozen by means of the freezing machine fastened to the microtome. The sections are dropped into water, transferred to 60% or 70% alcohol for one minute and then placed in water, where they flatten out and float. They are floated on a slide, and the section is covered with several drops of thionin for 30 seconds; it is then covered with a cover glass and the excess thionin blotted. Polychrome methylene blue, alkaline or acidulated with glacial acetic acid, may be used instead of thionin. Or the sections may be stained in small dishes filled with the stain. This will give a more uniform stain, but thin sections are liable to become torn. This method gives a rapid bedside diagnosis. If great haste is not necessary, the specimens may be fixed in a 5% formaldehyde heated at 40 degrees C. for 10 minutes, and then frozen and placed on a slide, the water blotted off, the sections covered with absolute alcohol and pressed to the slide. They are covered with a very thin celloidin solution, the excess drained off and the celloidin permitted to set but not to dry. As soon as the celloidin is set the slide is dipped in water, to wash away the alcohol and ether and then placed in Delefield's hematoxylin diluted 1 in 10 in water, for a few seconds until it is stained; wash in water until all excess stain has been washed away, then placed in a solution of alcohol or aqueous eosin (1 in 1,000 for a few seconds; washed in water or alcohol (according to the eosin solvent used) and transferred for 5 minutes to 95% alcohol; then placed in fresh alcohol until dehydrated and cleared with carbolxylol, or oil of organum crotici. In using the carbolxylol, care must be taken to wash out all the phenol with several baths of xylol, or it will decolorize the specimens. After dehydration a cover-glass is cemented on the specimen with Canada balsam, or a thick solution of damar in xyol.

STAINING THE SECTIONS

Sections from tissues fixed in Zenker's solution must have all the precipitated mercuric oxide removed by treating with Lugol's solution or a 1% alcoholic eosine solution for 10 to 20 minutes, and the iodine removed by washing with alcohol before staining.

Celloidin sections must be first placed in 95% alcohol, blotted and washed twice with xylol, or washed until clear with xylol.

THE STAINING METHOD

1. Stain in a 5% aqueous solution of eosin for 20 or more minutes.
2. Wash in water to remove the excess of eosin.
3. Stain in Unna's alkaline methylene blue solution, diluted 1 in 4 with water for 10 to 15 minutes. Unna's methylene blue is composed of methylene blue 1 part, sodium carbonate 1 part and water 100 parts.
4. Wash in water.
5. Differentiate and dehydrate in 95% alcohol, keeping the section in constant motion, so as to uniformly decolorize. Results must be controlled by frequent microscopical examination.
6. Wash in xylol.
7. Mount with xylol damar or Canadian balsam.

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